VOLUME 4 January 1955 NUMBER 1

THE AMERICAN JOURNAL OF

Tropical Medicine and Hygiene

Combining The American Journal of Tropical Medicine and The Journal of the National Malaria Society

OFFICIAL ORGAN OF
THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

PUBLISHED BIMONTHLY BY
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BALTIMORE 2, MARYLAND, U.S.A

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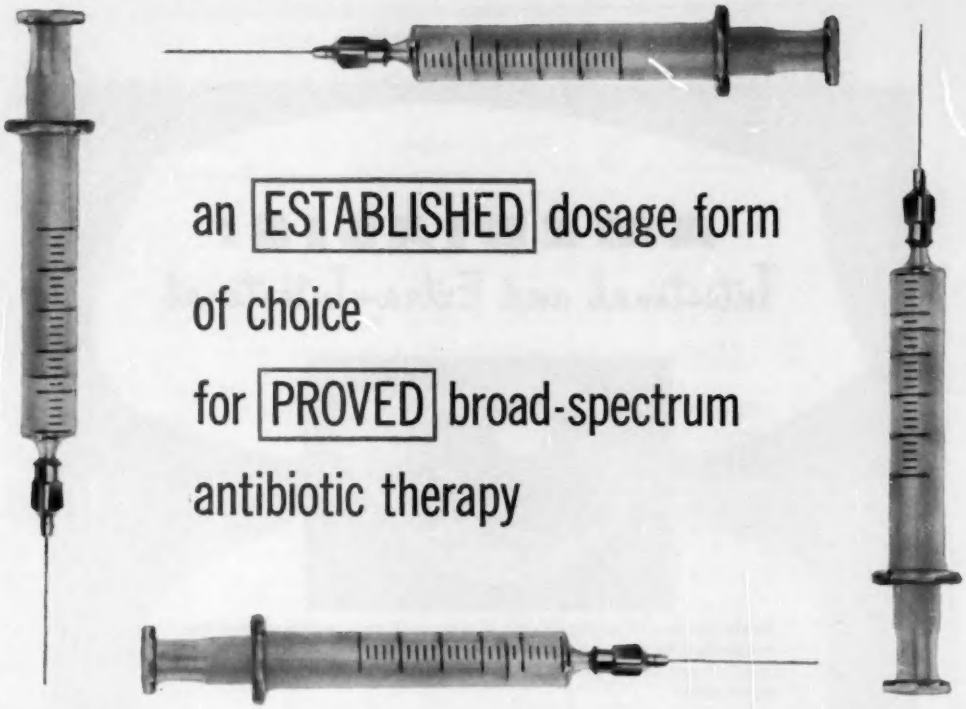

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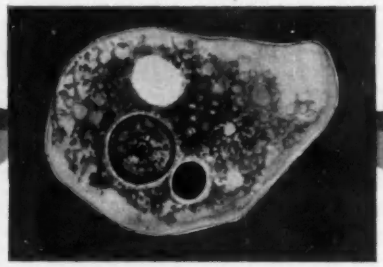

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1. Sodeman, W. A., and Denver, P. C.: *Am. Jour. Med.*, 12:440, Apr., 1952.
2. Zovals, D. C., and Hamilton, H. E.: *Ann. Int. Med.*, 36:110, Jan., 1952.
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Tropical Medicine and Hygiene

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Accepted articles for which immediate publication would seem important will be inserted in the next issue of the journal to go to press if the author is willing to pay the manufacturing cost. The insertion of such articles will not affect the publication of other manuscripts awaiting their turn, since the inserted articles will constitute additional pages to the volume without additional cost to the subscriber.

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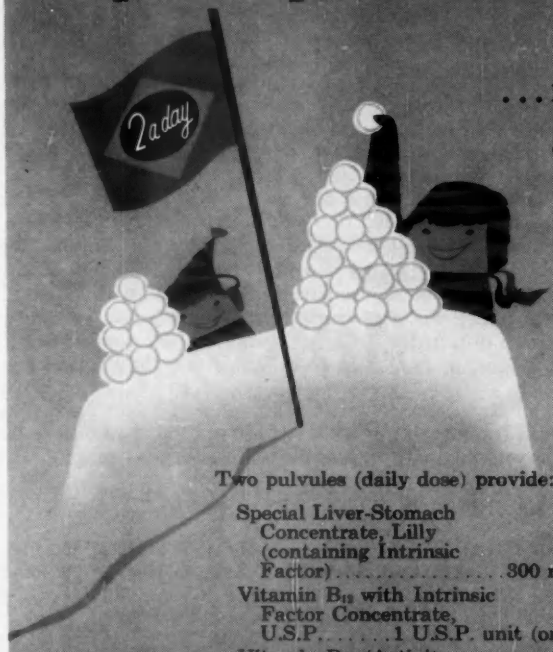

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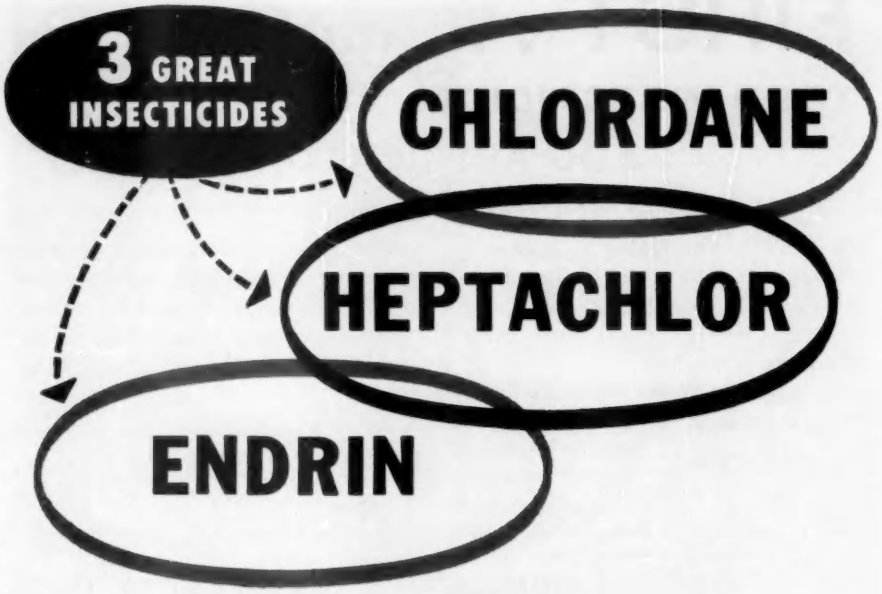

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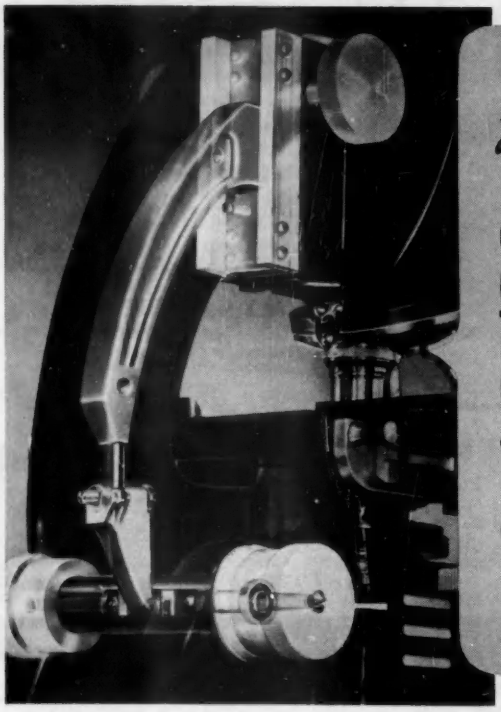

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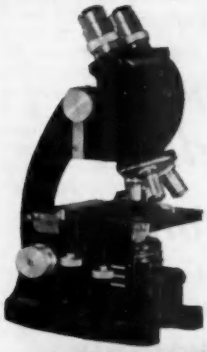

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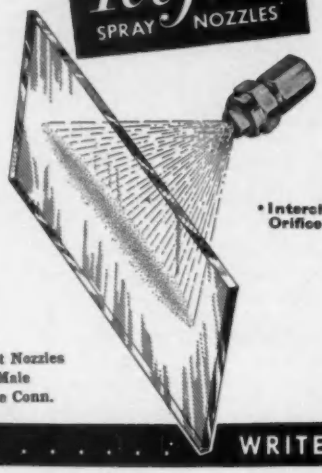

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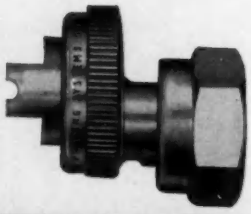

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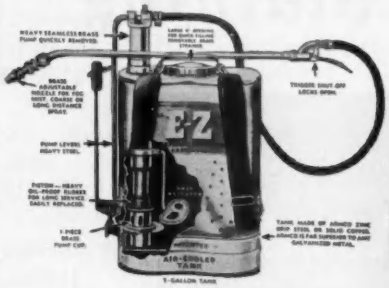

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THREE CASES OF HUMAN INFECTION WITH *ISOSPORA* IN GEORGIA

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Although human infections with the coccidium *Isospora* are usually considered to be rare, between three and four hundred cases have thus far been reported. Twenty-seven cases were found in the United States. Table 1 summarizes the available information concerning the cases found in this country including the three infections here reported.

Six of the eight cases found by Kofoid *et al.* (1919) and one reported by Kofoid and Swezy (1920) were in troops recently returned from overseas; since the patient examined by Magath (1935) came directly to the Mayo Foundation from Honolulu for medical treatment, the infection was thought to be of Hawaiian origin; the two cases described by May (1947) and MacCready *et al.* (1947), and the five reported by Markell *et al.* (1947) in naval personnel who were prisoners of the Japanese in the Philippines during World War II were almost certainly acquired in the Philippines; the two found during a survey of naval patients by Markell *et al.* (1947) probably had contact with persons who had been overseas, and the most recent infection reported was in a sailor returned to this country after three years service in Brazil (Wells and Bucki, 1953). Thus eighteen of the cases reported in the United States are very probably of extra-continental origin.

No record exists of the degree of association between two cases reported by Kofoid *et al.* (1919) and two by Kofoid and Swezy (1920) in World War I troops on home service who were food handlers at the port of debarkation in New York City, and the seven cases described by them in troops with overseas service. It is apparently possible that the cases were related. In addition, Liebow *et al.* (1948) have expressed doubt as to the authenticity of the case described from Ohio by Kiskaddon and Renshaw (1945) on the grounds that the photographs in the text did not demonstrate the parasite convincingly.

This leaves only the Tennessee case reported by Meleney *et al.* (1932), the background of which is unreported; the case from California which appears to have been related to an infection in the patient's cat (Kessel, 1933), and the two cases reported by Markell (1950), also from California.

Considering the circumstances surrounding all the above cases, it appears that the majority of human *Isospora* infections reported in this country were acquired in foreign countries and that some of the remaining cases were very probably acquired from contacts with persons who had become infected in foreign countries. Since human *Isospora* has been rarely recorded in this country, it seems worthwhile to describe three infections, the first to be reported from Georgia. The first two cases were discovered in the same family living on a farm near

TABLE 1

Human isospora *cases reported from the United States*

Author and Date of Report	Type of Investigation	Location	No. of Positives	Residence of Patient(s)	Time in United States	Presumed Location of Infection	Stage of Parasite
Kofoid *et al.*, (1919)	Survey of Military personnel	New York City	8	Unspecified	6 recent returnees from overseas duty	Overseas	Unspecified
					2 home service	Camp contacts?	Unspecified
Kofoid & Swezy. (1920)	Survey of Military Personnel	New York City	3	Unspecified	1 recent returnee from overseas duty	Overseas	Unspecified
					2 home service	Camp contacts?	Unspecified
Meleney *et al.*, (1932)	Survey of civilian personnel	Tennessee	1	Tennessee	Unspecified	Tennessee	Unspecified
Kessel, (1933)	Clinical Exam.	Los Angeles	1	Los Angeles	Unspecified	Los Angeles	Sporocyst
Magath, (1935)	Clinical Exam.	Mayo Clinic	1	Honolulu, T. H.	Few Days	Honolulu	Oocyst
Kisskaddon & Renshaw, (1945)	Clinical Exam.	Ohio	1	Ohio	Unspecified	Ohio	Sporocyst?
May, (1947)	Clinical Exam.	New Mexico	1	Unspecified	Returned from Philippines 3 months	Philippines	Oocyst
MacCready *et al.*, (1947)	Clinical Exam.	Colorado	1	Unspecified	Recently returned from Philippines	Philippines	Oocyst
Markell *et al.*, (1947)	Survey Naval Hosp. Pts.	California	2	Unspecified	Unspecified	Hosp. contacts?	Oocyst
	Survey of U. S. prisoners of war	California	5	Philippines	Recently returned from P. ilippines	Philippines	Oocyst
Markell, (1950)	Clinical Exam.	California	2	California	Unspecified	California	Sporocyst
Routh *et al.*, (Present report)	Clinical Exam.	Georgia	1	Georgia	Lifetime	Georgia	Oocyst
	Family of Patient		1				Sporocyst
	Survey		1				Sporocyst
Wells & Bucki, (1953)	Clinical Exam.	Bethesda, Maryland (U. S. Navy)	1	Brazil for 3 years	Returned 3 weeks after service in Brazil	Brazil	Oocyst

Reidsville, Tattnall County, while the third was found during a survey for amebiasis in Blakely, Early County.

DESCRIPTION OF CASES

Case 1. This case, a housewife, 39 years of age, exhibited persistent diarrhea from December 3, 1949, to January 5, 1950, with as many as six loose stools a day. The first stool to be examined was collected on December 24, and sent to the laboratory of the Georgia Department of Public Health with the request that it be examined for *Endamoeba histolytica*. This examination revealed no evidence of amebiasis; however, many *Isospora* oocysts were found. Oocysts were again found on December 31, January 3, 5, 10, 16, and 18. None could be demonstrated in seven subsequent stools submitted from January 23 through June 15, and it was assumed that the infection had been lost. The elapsed time from onset of symptoms to the end of the cyst passing period was 47 days. Parasites other than *Isospora* were not found in the stools examined and stool cultures made on January 3 and 18 were negative for pathogenic bacteria. The diarrhea is therefore believed to have been caused by the *Isospora*.

This case received large doses of colloidal kaolin, pectin, aromatics, opium, cremo-suxidine and bismuth subcarbonate without effect. After being put on large doses of bismuth salicylate, however, the diarrhea stopped almost abruptly. She had two stools on the first day of this treatment and only one each day thereafter. Thirteen days after beginning this treatment oocysts could not be found.

To determine if this patient's husband and three children were infected with *Isospora*, several stools were examined from each. Hookworm eggs were found in the stools from each of the children, and one of them, a daughter, 14 years of age, was found to be infected with *Isospora* (Case 2).

Case 2. This case exhibited no symptoms but on January 25 a stool was submitted in which mature *Isospora* sporocysts were demonstrated. No evidence of infection had been seen in previous stools collected on January 3, 5, and 18. On January 30, February 1, 6, and 9, mature sporocysts were again present, but in no instance were entire oocysts found. A stool collected on February 14 and four subsequent stools collected through February 23 were negative for *Isospora*. This infection, therefore, lasted at least 16 days or for the period during which sporocysts were passed. Since this case did not develop symptoms she was not treated.

Case 3. This case, which was discovered in Blakely while making an epidemiological survey for amebiasis, was a negro female, eleven years of age. She was said to be the only one of five children in the family who had recently complained of "bowel trouble". However, since she was also infected with *Hymenolepis nana*, the dwarf tapeworm, her symptoms cannot be ascribed without question to the *Isospora*. Four stools submitted over a period of 34 days, on January 20 and 27, and February 6 and 23, 1953, contained only mature sporocysts similar to those passed by Case 2. Stools from the mother and four other children in the family were negative for both *Isospora* and the dwarf tapeworm.

Source of infections. The probability of these cases having been acquired from a foreign source is remote since no member of the families had ever left this country nor had any of them visited in the homes of or otherwise been in close contact with persons recently returned from foreign countries. The possibility of the existence of a local host was therefore considered.

In seach for possible hosts feces were obtained from the family dog and several hogs and chickens, all of which were living on the premises of the family which included the first two human cases.

In the first stool collected on January 18, 1950, from the dog, *Isospora* oocysts, morphologically similar to those passed by Case 1, and mature *Isospora* sporocysts, similar to those passed by Case 2, were demonstrated. No oocysts or sporocysts were found on January 25, but on February 1 sporocysts, and on February 6 oocysts and sporocysts were again observed. No evidence of infection with *Isospora* was found in stools from seven hogs or in droppings from several chickens. On January 18, 1951, one year later, oocysts were again demonstrated in a stool from the same dog and at this time two other dogs living in the immediate neighborhood were examined and found to be free of the infection. Since both Case 1 and Case 2 had dressed squirrels just prior to the recognition of their infections, three of these animals were taken in the neighborhood when opportunity afforded one year later. All three were found to be negative for *Isospora* on January 18, 1951.

The source of the infection in Case 3 could not be thoroughly investigated. A neighbor's dog with which all five children played, and mice which commonly harbor the dwarf tapeworm, are to be considered as possible sources of the *Isospora* in this case.

An additional case in a dog. In April, 1953 the stool from a young dog which had recently been transported from New York to Atlanta was submitted to confirm a diagnosis of coccidiosis. There were present in this stool mature *Isospora* sporocysts which were morphologically similar to those found in Case 2, Case 3, and the dog described above. Sporocysts were passed in four stools collected on April 13, 17, and 28 and on May 5, 1953.

Laboratory studies. The character of the stools from the first two cases was similar to that previously reported in human *Isospora* infection. They were frequently soft, light in color, and contained numerous fatty acid and Charcot-Leyden crystals. A noticeable quantity of mucus was also found in the stools from the first case. The stools from the third case, however, were consistently dark in color, were well formed and no characteristic crystals were found.

A laboratory study of oocysts from the first case and from the dog showed that they usually contained an undifferentiated zygote when passed. After several hours the zygote divided into two sporoblasts, which after 20 to 24 hours, in most instances, developed into mature sporocysts, each containing four sausage shaped sporozoites. However, some oocysts which were refrigerated for one week, prior to development at room temperature, were found to contain only one large sporocyst with eight sporozoites.

The mature sporocysts obtained from Case 2, Case 3, and the dogs were found

either singly or in pairs. The measurements of oocysts and sporocysts from all three human cases and the dogs are shown in Table 2.

Two white mice and a guinea pig each were fed numerous mature oocysts from Case 1, but stool examinations over a period of 45 days and subsequent autospy showed no evidence of infection.

DISCUSSION

The oocysts from Case 1 and the first dog were similar to oocysts described from human *Isospora* infections by other investigators, and conform to the definition for *I. belli* as proposed by Elsdon-Dew and Freedman (1953). The definition which these investigators propose is stated as follows: *I. belli* (Wenyon 1923): "May be passed at all stages of development; immature forms mature in up to 5 days. Oocysts 30 μ x 12 μ. Sporocyst 11 μ x 9 μ. Typically no oocystic residual body; sporocystic residual body finely granular with limiting membrane, compact and centrally placed between the four sporozoites."

Magath (1935) reviewed the human *Isospora* cases reported prior to 1935 and gave the size range for oocysts described by numerous investigators (Table 2). These ranges and the derived averages are remarkably similar to those obtained for oocysts from Case 1 and the dog.

Reichenow (1925) first reported the presence of *Isospora* sporocysts not accompanied by oocysts in stools from a human infection; Kessel (1933) also observed sporocysts but not oocysts from a human case in California and from a pet cat belonging to the patient, and Markell (1950) reported sporocysts only from the two cases he investigated. More recently Meira and Corrêa (1950) found 15 cases and Elsdon-Dew and Freedman (1953) 23 cases in which only sporocysts were passed. Both Meira and Corrêa, and Elsdon-Dew and Freedman suggest that the sporocysts which they studied should be distinguished from *I. belli* and identify them as *I. hominis*. This species appears to be indistinguishable from *I. bigemina* found commonly in the dog and it is entirely possible that these two species are actually the same. The definition proposed by Elsdon-Dew and Freedman for *I. hominis* is stated as follows: *I. hominis* (Railliet and Lucet 1891): "Usually passed fully developed. Oocyst wall usually absent. Sporocysts may be single or coupled in pairs, each being 15 μ x 10 μ. Sporocystic residual body of coarse loosely aggregated granules appearing polar in position separate from the four sporozoites." The measurements of sporocysts reported by various investigators are recorded in Table 2. The sporocysts studies from Case 2, Case 3 and the two dogs conform to this definition. Although the average dimensions of sporocysts from the human cases are slightly smaller than those of sporocysts from the dogs they both fall within the same size range. Considering the small number of measurements made it would appear more accurate to identify the sporocysts on the basis of the internal morphological characteristics which conform to the definition for *I. hominis*.

While it appears reasonable to assume that the first dog was the source of the infections in Case 1 and Case 2, it would be equally logical to suppose that both dog and the human cases were infected from a common source. Several

investigators have previously expressed the belief that the human *Isospora* are identical with species in lower animals and are accidentally acquired by man. Magath (1935) mentioned that in regions where human infections are most common, both cats and dogs are reported to be frequently infected with *Isospora*, while Franchini (1927) suggested that the cat or dog was the probable source of two human infections which he studied, and Kessel (1933), as noted above, found sporocysts in the feces of a patient which were morphologically similar to those found in the feces of the patient's cat. Magath (1935) after reviewing the reports of attempts to infect some of the lower animals with human oocysts, states that Fantham (1917), who was able to infect kittens, is the only investigator who has been successful. There appears, however, to be some occasion to question the supposition that Fantham was using kittens initially free of *Isospora* in this experiment since several other investigators have been unsuccessful in attempting to infect kittens as well as various other laboratory animals with human oocysts.

Although our findings add weight to the assumption that human infections with *Isospora* may originate from infections in dogs, we feel that additional investigation is needed to determine if human beings can become infected with the *Isospora* obtained from dogs, or if some common source of infection exists for both man and dogs.

TABLE 2

*Measurements of oocysts and sporocysts**

Case	Number of Sporocysts or Oocysts Measured	Range in Length	Range in Breadth	Average Dimension
#1	10 oocysts	27.17–34.32	11.44–13.59	29.53 x 12.01
First Dog	10 oocysts	27.17–31.46	10.73–15.02	29.38 x 12.87
Magath (1935)	Oocysts from all human cases prior to 1935	21.75–40.00	10.00–19.00	29.00 x 13.00
#2	9 sporocysts	11.44–12.87	9.20–10.73	12.32 x 9.51
#3	6 sporocysts	11.66–12.90	9.30–10.42	12.29 x 9.95
First Dog	14 sporocysts	11.44–16.50	9.30–10.70	15.35 x 10.30
Second Dog†	7 sporocysts	12.08–14.99	8.33–10.83	13.51 x 9.70
Reichenow (1925)	2 sporocysts	16.00	10.50	16.00 x 10.50
Kessel (1933)	Sporocysts from human case	14.00–16.50	9.00–11.00	15.4 x 9.50
	Sporocysts from pet cat			Approximately the same size as those from human case
Meira & Corrêa (1950)	Sporocysts from 15 human cases	10.00–18.00	7.50–12.00	14.80 x 9.80
Elsdon-Dew and Freedman (1953)	Sporocysts from 23 human cases	14.00–16.00	9.00–11.00	15.00 x 10.00

* Measurements are in micra.

† This dog was not associated with the human cases.

A review of the literature discloses that a number of drugs have been used with varying results in the treatment of *Isospora* infection in man. It should be pointed out that many infections which have been followed were found to be self-limited or the production of oocysts to be variable from day to day, with periods when none were passed followed by their reappearance. Therefore, the disappearance of oocysts immediately following treatment may be coincidental. In this connection Magath (1935) found oocysts in only one stool from his patient although several were examined; Barksdale and Routh (1948, and unpublished data) observed four infections in which the passage of oocysts stopped although no treatment was given, and described a noticeable fluctuation in numbers of oocysts passed from day to day; the two experimental human infections described by Matsubayashi and Nozawa (1948) cleared up completely without treatment. Furthermore, sporocysts from the second human case in the present report spontaneously disappeared from the stools after three weeks although no treatment was administered. In the first case, since 13 days elapsed after treatment before oocysts disappeared from the stools, it is certain that bismuth salicylate treatment did not immediately eradicate the infection even though symptoms ceased after it was administered. Nevertheless, since our patient made a dramatic response to bismuth salicylate and several other investigators have also found this drug to be effective, it might be considered to have value in controlllng symptomatic *Isospora* infection in man.

SUMMARY

One case of human infection with *Isospora belli* and two with *I. hominis* are described from two counties in Georgia. All of the infections appear to have been acquired locally and two of them were apparently related to an infection in the family dog. Morphologically the species of *Isospora* found in the human cases was similar to that recovered from the dog, suggesting that two of the human infections might have originated from the dog. However, it is suggested that further investigation is needed to determine if humans can be infected with the *Isospora* obtained from dogs and if a common source of infection exists for both man and dogs. Since clinical symptoms subsided following the administration of bismuth salicylate in one of the cases, it is suggested that this drug may have value in controlling symptomatic *Isospora* infection in man.

ACKNOWLEDGMENTS

The writers are indebted to Dr. E. J. Sunkes, Director, and Miss Madge Reynolds, in charge of the Microscopy Unit, the Division of Laboratories, and to Dr. W. J. Murphy, Director of the Division of Epidemiology, all of the Georgia Department of Public Health, for valuable suggestions made during the preparation of the manuscript. The writers also wish to thank Mr. Ralph Ramsey, field biologist with the Division of Epidemiology, for assisting in the epidemiological investigations.

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ATABRINE IN HEPATIC AMEBIASIS

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After it was found that atabrine possesses a potent amebicidal action *in vitro* (Nor El-Din and El-Kordy, 1949; Radke, 1951) and inasmuch as it was already known that the drug attains high concentrations in the liver (Dearborn *et al.*, 1943; Army Malaria Research Unit, 1946b), it was but natural to try the drug in hepatic amebiasis.

Although atabrine has been used in intestinal amebiasis (Abd El-Ghaffar, 1948; Nor El-Din and El-Kordy, 1949; Radke, 1951), data on its use in hepatic amebiasis are both very scanty and incomplete. Spelberg and Zivin (1945) reported two cases of amebic liver abscess which received atabrine for malaria with temporary improvement in one and no improvement in the other case. Radke (1951) reported "an amazing clinical response" to atabrine in a case of amebic liver abscess which penetrated the diaphragm and formed a bronchopleural fistula, in which two courses of emetine in less than a month were ineffective. No mention was made however regarding the follow-up of the case.

The present paper reports the results of atabrine therapy in three cases of amebic liver abscess and one case of acute amebic hepatitis.

Case I. This was a big neglected liver abscess in a man of 45 years. Atabrine was given in doses of 0.1 gm. t.d.s. for 10 days and meanwhile three aspirations were done at intervals of a few days. The first aspiration brought 1200 cc. of chocolate pus, the second 200 cc. and the third only few cc. No antibiotic was given. The man was discharged apparently cured, but two months later he returned with a right chest empyema. It was thought that the relapse in this case might have been due to the use of a relatively small dose and it was decided to treat the subsequent case with a bigger dosage.

Case II. The case arrived in a moribund state with a huge fluctuating liver swelling. There was extensive generalized edema and a pronounced anemia. The pulse was rapid and feeble, the blood pressure was 80/60 and the temperature was not elevated. Aspiration brought about 2 liters of chocolate pus and a very foul-smelling gas, and 4 more liters were removed through open drainage. Atabrine was given in doses of 200 mg. t.d.s. for 15 days, together with supportive therapy. No other amebicide was given and, of the antibiotics, only penicillin and streptomycin were used. The patient made an uneventful recovery and by the end of one and a half months the wound was perfectly healed and the patient could resume his work.

Six weeks later, the patient returned with a discharging sinus in the 7th intercostal space in the right lower axillary area. A radiograph taken after injection of lipiodol showed a cavity 8 or 9 cm. in diameter and 11 cm. deep. Fluid from the sinus was yellowish and contained no amebae. The general condition of the patient remained, however, quite good.

Case III. This was a case of acute amebic hepatitis in a man of 40, in whom a misdiagnosis of acute cholecystitis had at first been made and penicillin and streptomycin given for a few days without producing any improvement. Screening showed that the right cupula of the diaphragm was raised and immobile. Atabrine in doses of 0.9 gm. daily for 7 days brought about a rapid recovery. Follow-up of the case for 6 months showed no relapse.

Case IV. In this case, a man of 50, there was a very big liver swelling which encroached on the lower abdomen and from which chocolate pus was aspirated. The general condition was bad and there was massive edema of the legs and scrotum and a severe degree of anemia. Treatment was conducted as follows:

1. Varidase (Streptokinase and Streptodornase, Lederle) was injected and 6 hours later, all pus was aspirated and 10 cc. of 1:100 solution of atabrine instilled. This process was repeated every fourth day.
2. Atabrine was given orally in doses of 0.3 gm. t.d.s. for 10 days.
3. Adjuvant therapy in the form of vitamins, blood transfusions, penicillin and streptomycin was given.

Improvement in both the general and local conditions was progressive and steady. Starting with 2500 cc. of pus on the first aspiration, only a few cc. of bright yellow clear fluid were obtained on the last aspiration done 25 days later. At the time of discharge the right cupula was moving quite freely and the thymol turbidity, thymol flocculation and cephalin cholesterol flocculation tests disclosed only a slight impairment of liver function. This case has been followed for the last 6 months with no evidence of relapse.

IN VITRO WORK

This was undertaken to compare the amebicidal efficiency of atabrine, chloroquine and emetine.

Method. We used a strain of *Endamoeba histolytica* which had passed about 500 forty-eight hour transfers in association with a mixed bacterial flora. It was kindly supplied by U. S. Naval Medical Research Unit No. 3, Cairo.

At first we used Difco bacto endamoeba medium in diluted horse serum, but later found that a medium of solidified horse or camel serum in diluted serum of the same animal gave equally satisfactory results. Although the growth starts slowly on the latter medium, the viability is more prolonged than on the Difco medium. To both media, minute amounts of finely powdered rice starch were added just before inoculation. To the tubes of media inoculated with *E. histolytica*, the drugs were added in serial dilutions, the tubes incubated at 37°C. and the results read 24, 48 and 72 hours later. Results indicated that atabrine is about 10 times as effective as chloroquine but only two-fifths as effective as emetine. Our results differ from those of Nor El-Din and El-Kordy (1949), using horse serum-Ringer medium, who found that atabrine is one-tenth as effective as emetine, and from Radke (1951), employing egg slant and liver infusion cultures, who concluded that the effect of atabrine was of the same order as that of emetine.

ADDENDUM

Since this paper was written, the sinus which developed in Case II after apparent cure with antibiotics was treated by instillation of 10 cc. of one per cent atabrine solution locally into the cavity every third day. After several treatments the sinus closed and now, one month later, the liver is but slightly enlarged, the diaphragmatic cupula moves freely, and the patient is in good general condition, having gained 4 kgs. in weight. Thus atabrine may be considered to have failed in Case I only, due probably to inadequate dosage and failure to institute local therapy with the drug.

DISCUSSION

Berliner *et al.* (1948) showed that the concentration of atabrine in the liver reaches about 10 times that of chloroquine after 10 days administration of each. On the basis of these considerations, atabrine appears to be more favorable than chloroquine in hepatic amebiasis.

In our own *in vitro* studies we found that the minimal effective amebicidal concentration of atabrine is 1:5,000. To attain this concentration in a normal adult liver weighing about one and one-half kilos would require the presence of about 0.3 gm. of the drug in the organ. Annegers *et al.* measured the atabrine content of the livers of dogs given oral doses of atabrine and killed at intervals. From their results it can be estimated that the above amount would accumulate in the liver after a total dose of 5 gm. of the drug has been ingested, which could be secured by means of a dose of 0.9 gm. daily for 6 days. Allowing for an extra period of 48 hours for the drug to act, the minimal time of administration would be 8 days. A larger quantity will obviously be needed to produce an effective concentration in a liver which has gained weight as a result of a hepatitis or an abscess. While in hepatitis it may be generally possible to attain the necessary concentration through oral therapy, in abscess, where the liver might sometimes reach enormous dimensions, local instillation of a strong solution of atabrine (e.g. 10 cc. of 1:100) into the abscess cavity after aspiration of the pus, appears necessary in many cases in order to achieve an effective concentration of the drug in the organ. This procedure should, in our view, be practiced in every case of abscess of moderate or large size. Failure in Case I, as well as in Spelberg's case (1945), might possibly have been obviated if this local therapy had been adopted.

A criticism which might be leveled at the above calculation is that it presupposes a parallelism between the concentration required for *in vitro* and *in vivo* action. Although such a parallelism may not always follow, Ball (1946) found in the case of *P. knowlesi* that the same concentrations of quinine and of atabrine are required to suppress growth *in vivo* and *in vitro*.

In the present study the following observations have also been made: 1) atabrine in doses of 0.9 gm. daily for 10 days was not toxic to the liver since in Case No. 4 after this dosage there was only a slight impairment in the thymol turbidity and cholesterol cephalin flocculation tests, due, in all probability, to

the abscess itself rather than to the drug; 2) the value of streptokinase in liquefying the thick pus of the abscess was demonstrated in Case No. 4.

SUMMARY

Atabrine cured a case of acute amebic hepatitis which received the drug orally, and a case of advanced amebic abscess of the liver to which, besides oral therapy, intrahepatic instillations of the drug were given. It failed to cure two other cases of advanced amebic liver abscess which received the drug by the oral route only. Inadequate dosage in one of the two latter cases, and secondary infection in the other probably contributed to the failure. *In vitro* studies on *Endamoeba histolytica* showed that atabrine is about 10 times more effective than chloroquine and about 40 per cent as effective as emetine. A dosage schedule for atabrine in hepatic amebiasis has been set up on a purely tentative basis.

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FIRST REPORT ON THE TREATMENT OF SLEEPING SICKNESS WITH PUROMYCIN

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The discovery of antibiotics opened up an extensive field for the search of new therapeutic weapons against both human and animal trypanosomiases since the available chemotherapy did not solve all the problems of the treatment of these diseases, especially those arising from drug resistance. Also, ease of administration and the low toxicity demonstrated by most of the antibiotics have encouraged their trial. In fact, almost all the known antibiotics have been tried out, but unfortunately none of them has fulfilled expectations. A new antibiotic, Puromycin,[1] recently produced from *Streptomyces albo-niger*, has shown activity against Trypanosomidae, opening a new field of laboratory and clinical research.

Puromycin has only a narrow spectrum against bacteria but, as shown by Porter *et al.* (1952), has a remarkable effect upon *Trypanosoma equiperdum* as evidenced in laboratory work with rabbits and mice, and moreover possesses a certain inhibiting power against *Trypanosoma cruzi*. According to these investigators, when mice were infected with *T. equiperdum* 2–6 hours before administration of the drug, a total dosage of 70–140 mg./kg. by intraperitoneal route was required to stop the infection, divided into 7 daily doses of 10–20 mg./kg. If treatment was started 24 hours after infection a total dosage up to about 105 mg./kg. (15 mg./kg. a day for 7 days) was ineffective, and if the treatment began 48 hours after the infection, a total dosage above 280 mg./kg. was necessary to check the infection. Experimental treatment with a single dose has shown the product to be active when 400 mg./kg. were administered intraperitoneally. Orally, the efficient dosage was a little larger: 140–210 mg./kg. (20–30 mg./kg. a day for 7 days). In rabbits similar results have been obtained by the intravenous and intramuscular routes.

The effects of Puromycin on *T. cruzi* were studied both *in vitro* and *in vivo*. *In vitro*, it showed a trypanocidal action in dilutions greater than 1:40,000. *In vivo* the effects upon *T. cruzi* were less marked than upon *T. equiperdum* and the results were rather inconstant, encouraging in some series and disappointing in others, although identical doses were employed in both. The drug was ineffective against the tissue stages of *T. cruzi*. Porter *et al.* (1952) state that Dessau has seen nephritic symptoms caused by the antibiotic when administered parenterally to mice in doses of 25–100 mg./kg. during four weeks.

These results, though somewhat conflicting, induced us to investigate the activity of Puromycin on *T. gambiense*. After a preliminary series of experiments

[1] The Puromycin employed in this study was very kindly supplied by the Lederle Laboratories.

in which we were able to prove *in vitro* the susceptibility of this trypanosome to the antibiotic in dilutions of between 1:15,000 and 1:30,000, we made what we believe to be the first trial of the drug in the treatment of human sleeping sickness, the results of which are reported below.

MATERIAL AND METHODS

The drug was administered to 15 sleeping-sickness cases selected in Portuguese Guinea, none of them having had any previous specific treatment. Table 1 reports their sex, apparent age and tribe, with a summary of the laboratory tests confirming our diagnosis. In accordance with our routine procedures, sleeping-sickness patients were picked by gross clinical signs in periodic examinations of the natives of Portuguese Guinea and then subjected to the following laboratory tests:

1. *Lymph gland puncture.* Five slides were examined by five different observers. From patients with positive lymph gland puncture, those with more than a single hypertrophic gland were selected in order to have several glands to puncture during the control of therapy because, as Moura Pinto points out, punctures of the same gland made on consecutive days may give variable results since microhemorrhages can produce local trypanolytic phenomena responsible for negative results after the first puncture.

2. *Blood cultures.* Since Pinto (1952) and Pinto *et al.* (1952) were able by means of blood cultures or bone marrow cultures easily to isolate *T. gambiense*, and Neujean and Evans (1952) recommend this technique for the control of relapses, we have used them to evaluate the efficacy of Puromycin. We employed Weinman's (1947) medium prepared according to Henraard and Peel's (1950) modification. Blood donors for the preparation of this medium were selected from among volunteers whose blood had been previously shown to be favorable to the multiplication of the trypanosome. Six cultures were incubated for 10 days at 26–28°C. for each patient and were then examined by three independent observers. If this first examination was negative it was repeated daily for some 15 days.

3. *Lumbar puncture.* This was performed on every patient and the cerebrospinal fluid (Ferreira *et al.*, 1950) examined for proteins (Sicard and Cateloube's rachialbuminimeter) and for cells (Nageotte's chamber).

4. *Thick drop of blood.* In some cases 3 to 5 thick drops of blood were examined under cover-slip. The results of these examinations were so irregular, however, that we abandoned them.

5. *Liver and kidney tests.* Since in the beginning we had no knowledge of the toxicity of the drug, we took precautions to control its side effects, carrying out urine examinations for proteinuria and cylindruria, blood urea determinations and the bromosulfalein test; in addition, total and differential blood cell counts were made and the sedimentation rate investigated. These tests served not only to confirm the diagnosis, but to control the results of the treatment.

6. *Doses and way of administration of Puromycin.* The antibiotic was administered orally in capsules containing 250 mg. according to varied therapeutic

TABLE 1

Results of treatment of 15 sleeping-sickness patients with Puromycin

No.	Sex	Age	Tribe	Cerebrospinal Fluid		Duration of Treatment	Total Amt. of Drug Rec.	Examination of Gland Juice						Blood Cultures					
				Cells	Proteins			Before treatment	After treatment					Before treatment	After treatment				
									24 hr.	48 & 72 hr.	At end of treatment	10th day	15th day		24 hr.	72 hr.	At end of treatment	10th day	15th day
						days	*gm.*												
1	F	35	Papel	3	0.24	10	21.0	+	?	−	−	−	−	+	?	−	−	−	−
2	F	25	Papel	4	0.20	7	13.5	+	+	−	−	−	−	+	+	−	−	−	−
3	F	30	Papel	25	0.25	7	13.5	+	+	−	−	−	−	+	+	−	−	−	−
4	F	45	Papel	35	0.40	7	13.5	+	−	−	−	−	−	+	−	−	−	−	−
5	F	20	Papel	4	0.20	7	13.5	+	−	−	−	−	−	−	−	−	−	−	−
6	F	17	Papel	6	0.22	7	13.5	+	+	−	−	−	−	+	+	−	−	−	−
7	M	25	Papel	7	0.24	7	13.5	+	+	−	−	−	−	+	−	−	−	−	−
8	M	20	Papel	2	0.22	7	13.5	+	+	−	−	−	−	+	−	−	−	−	−
9	F	30	Papel	10	0.22	7	13.5	+	+	−	−	−	−	−	−	−	−	−	−
10	M	40	Manjaco	101	0.22	7	11.5	+	−	−	−	−	−	+	−	−	−	−	−
11	M	30	Manjaco	25	0.18	7	11.5	+	−	−	−	−	+	+	+	−	−	+	+
12	M	15	Papel	42	0.40	10	9.5	+	+	−	−	−	−	+	+	−	−	−	−
13	M	20	Papel	2	0.22	10	9.5	+	−	−	−	−	−	−	−	−	−	−	−
14	F	35	Papel	15	0.24	10	9.5	+	+	−	−	−	−	−	−	−	−	−	−
15	F	20	Papel	10	0.20	10	9.5	+	+	−	−	−	−	+	−	−	−	−	−

schemes covering 7 to 10 days, the maximum daily dose ranging from 1.0 gm. to 2.25 gm., and the total amount received, from 9.5 gm. to 21.0 gm. Daily doses were fractioned into partial doses given at regular intervals of 8 hours or 12 hours. As will be seen, these variations made little difference in the results.

RESULTS AND COMMENT

From Table 1 it can be seen that in 5 cases, trypanosomes could not be found in gland juice 24 hours after treatment was begun and that in every other patient they disappeared from gland juice within 48 hours.

Blood cultures (Table 1) were similarly negative in a short time. Twenty-four hours after beginning the treatment, 9 patients had negative blood cultures and only Case 11 had by that time a negative gland puncture but a positive blood culture; 72 hours after starting the treatment all blood cultures were negative.

Treatment was stopped on the 7th–10th day. No other specific therapy was administered afterwards except in the two cases which relapsed and which were then submitted to the classical course of therapy. Of these two patients, Case 11 ran away after the second control on the fifteenth day, while Case 12 had a positive blood culture by the 60th day, though still with negative gland juice. Both had had small total doses of Puromycin (11 gr. in Case 11 and 9.5 gr. in Case 13). In both cases also the cerebrospinal fluid disclosed an aggravation of symptoms at the last examination. It seems worth mentioning that Case 11 had very numerous trypanosomes in the peripheral blood at the first examination and that in Case 12, lymph gland juice was exceptionally rich (5–6 trypanosomes per field, 210×).

Allowance being made for these two therapeutic failures, the results seem promising, though of course no definitive conclusion about the real value of the drug can yet be drawn. Patients who did not relapse continue under observation and without other specific therapy, and we expect to report later on their subsequent history. In the meanwhile these first results seem interesting enough to warrant publication.

Toxic manifestations attributable to Puromycin did not go beyond occasional headache, nausea and vomiting and a sometimes troublesome diarrhea (4–5 stools daily). All this stopped when treatment was interrupted.

ADDENDUM

Since the preparation of the manuscript patients have been controlled twice, viz. 3 and 6 months after treatment. The same methods of investigation were employed. Two more patients (Cases 4 and 10) relapsed; others did not show symptoms of the disease and had negative blood cultures.

SUMMARY

The results of the treatment of 15 cases of sleeping sickness by Puromycin are reported. Two months after the end of treatment, only two patients had relapsed; these were severe cases and had received relatively small doses of the antibiotic. Two other cases subsequently relapsed. Patients who did not relapse are under observation and will not receive any other specific therapy unless some

new relapse is discovered. These patients are periodically controlled by lymph gland puncture, blood culture and cerebrospinal fluid examination. Toxic signs of the antibiotic were unimportant, taking the form mainly of diarrhea.

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A REPORT OF A CASE OF IMPORTED VISCERAL LEISHMANIASIS OF A DOG IN THE UNITED STATES[1]

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The following report is the first in the United States of a case of visceral leishmaniasis in the dog.

HISTORY

The dog, a Doberman Pinscher, was born May 30, 1951, in Pfungstadt, Germany. The bitch and litter were kept in a barnyard which was reasonably clean, and all seemed in excellent health. In July, 1951, the dog was shipped to Greece and after a period of 6 months developed a skin condition thought to be due to dietary deficiency. The owner described these lesions as localized "open sores" on the dog's back. Close clipping of the hair and local medicinal applications did not effect a permanent cure. On the premises, which were not very clean, there were also 2 other dogs, pigeons, and rabbits. The climate in the summer was hot, and insects were very prevalent.

The animal remained in Greece until July 18, 1953 (2 years), when he was moved via boat to Boston, Massachusetts. While on the boat a Saluki from Arabia bit the Doberman in the right hock region. This leg swelled and had to be treated aboard ship with penicillin. The leg was treated in Boston and responded well. The owner stated that the dog never seemed to be the same after this bite wound.

The dog was shipped to Florida shortly after arrival in the U. S. A. In October he injured his right front leg by running into a wire fence. Three weeks later the dog was presented to the small animal clinic at the Alabama Polytechnic Institute for treatment of a lameness that appeared periodically in different legs.

CLINICAL DESCRIPTION

A. First period of hospitalization: November 10–21, 1953

Upon entrance to the clinic in Auburn, Alabama, November 10, 1953, the following symptoms were noticed. Muscle soreness was observed especially in the right front leg, but at other times tenderness was noticed in other legs. Affected joints and legs were radiologically negative. The temperature (during the 11 day period of hospitalization) varied from 101.6 to 104.8° F. Oral mucous membranes were slightly pale. The laboratory results were as follows:

RBC—6,153,000
WBC—10,300 (N—82%, L—12%, M—2%, E—4%)
Hb.—17.5 Gm.
Blood Urea Nitrogen—8.79 mgm. %

[1] Approved by the Committee on Publications, School of Veterinary Medicine, Alabama Polytechnic Institute; Publication No. 436.

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Sed. Rate—0.4 mm.—30 min.
1.0 mm.—60 min.
Serum Ca—13 mgm.%
Serum P—2.9 mgm.%
Brucellosis agglutination test—negative
Blood Culture—negative
Synovial fluid culture—negative
Fecal Examination: Giardia (cysts and trophozoites)—plentiful
No parasitic eggs were seen on flotation.

No definite diagnosis was made. It was reasoned that possibly a stubborn chronic generalized arthritis and myositis had occurred from the previous bite and joint infection. A tentative diagnosis of rheumatoid arthritis was made, and the dog was placed on chlorotetracycline (Aureomycin) and supportive vitamin therapy for 2 weeks. He was also vaccinated against rabies. The local veterinarian (Florida) followed the above treatment with cortisone acetate injections and reported that the dog seemed markedly improved.

B. Second period of hospitalization: February 17–March 15, 1954

The dog had been losing considerable weight and had been showing weakness and lameness for 3–4 weeks. During this time the left hind leg was most severely affected. A persistent fever of 103.0°F. was noted during this period.

Upon clinical examination, the oral mucosa was pale and slightly icteric, and the popliteal lymph nodes were enlarged. The dog had lost considerable weight, and was very emaciated. The laboratory results were as follows:

RBC—2,500,000
WBC—15,300 (N—88%, L—11%, M—1%, E—0)
Hematocrit—22%
Hb.—8.5 Gm.
Blood Urea Nitrogen—11.72 mgm.%
Sed. Rate—6.9 mm.—30 min.
7.2 mm.—60 min.
MCH—34; MCV—88
No protozoa were seen on numerous blood smears.
Icterus Index—8.5 units
Bromsulfalein—20% retention

A radiograph of the abdomen revealed an enlarged liver. The leg radiographs were negative.

On exploratory laparotomy the liver was observed to be enlarged and mottled. The spleen was enlarged due possibly to pentobarbital sodium anesthesia. A small liver biopsy specimen was taken. Subsequent treatment with oxytetracycline (Terramycin), choline, methionine and liver extract produced a temporary improvement in the animal's condition.

The material from the liver biopsy was cultured on routine bacteriological media with negative results. Histological examination of a liver section stained with hematoxylin and eosin showed that many Kuppfer cells were distended with

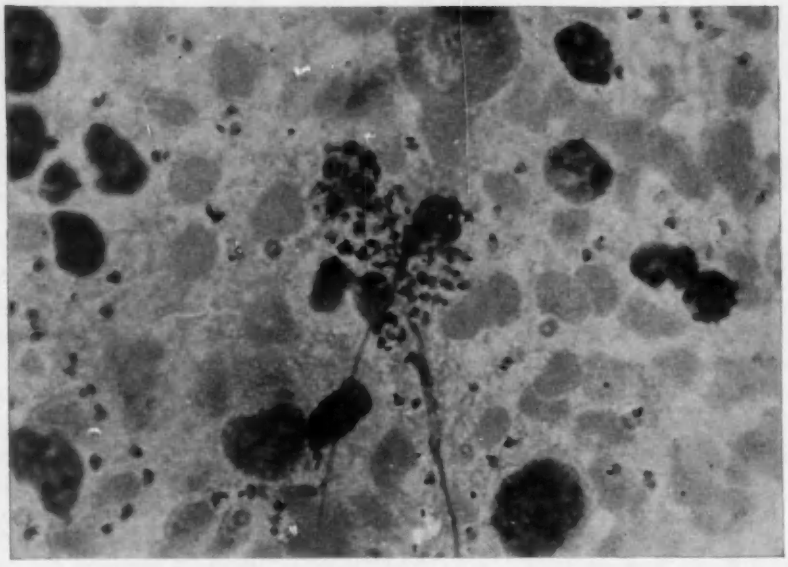

FIG. 1. Impression smear of spleen of dog showing numerous *Leishmania* organisms. Stained with Giemsa. Magnification about 1100×. Photomicrograph by D. H. Copeland.

numerous round bodies. Morphological characteristics of these bodies were more suggestive of *Leishmania* than *Histoplasma* or *Toxoplasma*. Critical study, including application of Bauer's stain for capsular material and Giemsa stain for structural detail, strengthened the hypothesis that the organisms were *Leishmania*. A diagnosis of leishmaniasis was made.

POST-MORTEM-EXAMINATION

Since the owner desired no further expense in the treatment of the dog, euthanasia was performed. The anterior lobes of the lungs were congested and consolidated; the lymph node at the thoracic inlet was enlarged. The hepatic and gastric lymph nodes were congested and enlarged. The liver was enlarged and mottled, and the cut surface showed some greenish-yellow discoloration. The kidneys showed several pitted areas or scars from previous inflammation. No other remarkable changes were seen. The spleen was normal.

Impression smears of the spleen and liver were stained with Giemsa, and many *Leishmania* organisms were found (Figure 1).

Cultures were made from the spleen and liver in the following media:

a) Fresh rabbit blood, reconstituted Difco-Bacto-tryptose agar and antibiotics*.
b) Fresh dog blood, reconstituted Difco-Bacto-tryptose agar and antibiotics.
c) Bovine serum, bovine hemoglobin, reconstituted Difco-Bacto-tryptose agar and antibiotics.

* Crystalline Penicillin G.—10,000 Oxford units per ml. culture medium. Streptomycin sulfate—0.5 mgm. per ml. culture medium.

These cultures were incubated at 22°C. Forty-eight hours later the cultures were all teeming with leptomonad forms of *Leishmania*.

The yolk sacs of six 5-day-old chick embryos were inoculated with 0.2 ml. of a suspension of liver and spleen (Wallace and Hamilton, 1946). The eggs were incubated at 22°C. Four days later several of the eggs were opened, and leptomonad forms were readily demonstrated in saline smears from the yolk sac.

Histopathologic examination was made of the liver, spleen, kidney, lung, gastric and hepatic lymph nodes, small intestine, skin, heart, and brain. The liver showed scattered large foci of inflammatory reactions consisting of mononuclear phagocytes and lesser numbers of lymphocytes and polymorphonuclear leucocytes. The cytoplasm of some of the mononuclear phagocytes contained numerous bodies morphologically characteristic of *Leishmania*. The inflammatory foci were located both in the hepatic lobules and to a lesser extent in the portal areas. No necrotic tissue was seen, but liver parenchyma within the inflammatory foci showed evidence of progressive atrophy and loss of cells. High power (430 ×) examination of the liver tissue outside the inflammatory foci revealed *Leishmania* within many swollen Kupffer cells throughout the lobules and in scattered mononuclear phagocytes in the periportal connective tissue. Many of the bile canaliculi in the central portion of the hepatic lobules were distended with bile.

Another marked pathologic change not necessarily connected with the *Leishmania* infection was also seen in the liver. At the periphery of the lobules a considerable amount of pale-staining hyaline material was deposited between the walls of the sinusoids and the hepatic cells. This material was in the characteristic location of amyloid and had caused marked atrophy of the adjacent liver cells, but it did not give the characteristic coloration of amyloid when stained with crystal violet or congo red.

Sections of the spleen did not show any inflammatory foci as seen in the liver. The white pulp was relatively inconspicuous, and the red pulp contained fairly numerous, diffusely distributed large mononuclear cells with *Leishmania* in their cytoplasm. The capsule of the spleen was infiltrated with small numbers of parasitized mononuclear phagocytes.

Evidence of the infection seen in the kidney tissue consisted of the presence of very occasional parasitized large mononuclear cells in the perivascular connective tissues. One glomerulus was found that also contained a parasitized large mononuclear cell. No other significant changes were seen.

The lung tissue also failed to show any significant inflammatory reaction. The only evidence of the infection in the lungs was the presence of occasional *Leishmania*-laden histiocytes in the alveolar walls. One of the three lung sections examined showed areas of acute secondary suppurative bronchopneumonia.

The sections of gastric and hepatic lymph nodes showed considerable proliferation of histiocytes in the cortical areas and diminution of the normal number of lymphoid elements. Many of the histiocytes contained *Leishmania*. The medullary sinuses were distended with large reticulo-endothelial cells containing considerable hemosiderin and a few *Leishmania*. The capsule and pericapsular connective tissue of one lymph node was heavily infiltrated with mononuclear phagocytes containing *Leishmania*.

The mucosa of the small intestine appeared to be free of the protozoa. However, at the junction of the internal and external muscular layers of the intestinal wall the tissue was moderately infiltrated with *Leishmania*-laden mononuclear cells.

The skin showed no appreciable inflammatory reaction, but on high power microscopic examination parasitized mononuclear phagocytes were found without difficulty along the course of the blood vessels and in the dermis apart from the vessels.

No lesions and no *Leishmania* were seen in the myocardium. The brain tissue and meninges also were devoid of cell reaction or *Leishmania*, but a few parasitized mononuclear phagocytes were seen in the connective tissue of the chorioid plexus of the fourth ventricle.

An abnormally heavy deposit of hemosiderin was found in the liver, spleen, and lymph node sections.

In summation, the basic histopathologic alteration was an invasion of the cells of the reticulo-endothelial system by organisms morphologically characteristic of *Leishmania donovani*. Parasitized cells of reticulo-endothelial origin were found in sections of the liver, spleen, kidney, lung, lymph nodes (gastric and hepatic), skin, and chorioid plexus of the fourth ventricle. Appreciable increase of reticulo-endothelial cells to form obvious proliferative or infiltrative lesions was observed only in the liver and lymph nodes and to a lesser extent in the muscular coat of the small intestine. The extent of parasitism and tissue reaction was less than that described in typical fatal human cases of leishmaniasis, but the basic histopathologic alterations were altogether characteristic of this disease.

DISCUSSION

This is the first reported case of visceral leishmaniasis in the dog in the United States. The infection apparently was not autochthonous but imported, presumably from Greece. During the animal's life in this country he was taken to Florida —one of the few places in the United States where possible vectors, flies of the genus *Phlebotomus*, are found. It is conceivable that the disease could have been spread in areas where these flies are found. Consequently, it seems that specific examination of dogs being imported into the United States from areas where leishmaniasis is endemic might be desirable. In view of the statement by Richardson (1948) that skin scrapings are reasonably reliable in detecting canine infections and the presence of the organisms in the skin in this case, it is suggested that skin biopsy may be an effective method of diagnosis, and a more practical one than the commonly recommended spleen, liver, or bone marrow biopsy.

ACKNOWLEDGMENT

The authors wish to thank Dr. Aubrey Robinson, Ft. Walton, Florida, for his interest and cooperation in referring this case to A.P.I. clinic.

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A NEW CONCENTRATION TECHNIC FOR THE DEMONSTRATION OF PROTOZOA AND HELMINTH EGGS IN FECES[1]

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The M.I.F. (merthiolate-iodine-formaldehyde) preservative stain developed by Sapero and Lawless (1953) for demonstration of protozoa and helminth eggs in feces has been in constant, extensive use by the writers for over a year. The method has proved to be especially reliable and rapid for hospital-ward patients and village stool surveys for protozoa, but need has been felt for even further improvement to enhance the usefulness of the technic as a combined protozoa and helminth-egg demonstration tool for large scale work.

To increase the diagnostic yield of the M.I.F. technic for helminth eggs and to retain its usefulness as a preservative, rapid stain, and diagnostic aid for protozoa, a concentration technic, referred to as M.I.F.C. (merthiolate-iodine-formaldehyde-concentration) has been developed in this laboratory. Comparison of the usefulness of the M.I.F.C. technic with M.I.F. vial technic direct smear, and four standard concentration methods commonly used in laboratories has indicated some advantages of the new technic.

MATERIALS AND METHODS

During the winter months (December to February, 1953–4) one hundred freshly passed fecal specimens were collected from Egyptian farmers living in Sindbis, a typical village located in the Nile Delta, northeast of Cairo. Specimens were taken without reference to age or sex, since they were to be used only to compare the M.I.F.C. method with other known technics. Fecal specimens were two to seven hours old upon receipt in the laboratory. Stool consistency varied from liquid to formed firm, but the majority were formed, soft and from gross appearance could be considered as normal stools. A maximum of ten minutes was allowed for the examination of a film made by any of the methods listed below. This is the usual amount of time devoted to examination for protozoa in many clinical laboratories.

The following technics (hereafter referred to by number) were tested:

1. M.I.F. stain preservative technic (Sapero and Lawless, 1953)
2. Brine flotation (Willis, 1921)
3. Zinc sulfate centrifugal flotation (Faust *et al.*, 1939)
4. Formalin ether sedimentation (Ritchie, 1948)
5. Acid ether sedimentation (Telemann, 1908)
6. M.I.F.C.

[1] The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

M.I.F.C. (MERTHIOLATE-IODINE-FORMALDEHYDE-CONCENTRATION) TECHNIC

This procedure employs the M.I.F. solution as a preservative and stain, with the addition of ether to dissolve fats and to float fecal detritus. The preserved fecal specimen is prepared as described by Sapero and Lawless (1953). Thereafter:

1. Mix the M.I.F. preserved specimen by shaking vigorously for five seconds.
2. Strain this mixture through two layers of wet surgical gauze into a 15 ml. centrifuge tube.
3. Add four c.c. of ether to the centrifuge tube, insert a rubber stopper, and shake vigorously. (If ether remains on top after shaking, add one c.c. of tap water and reshake). The ether used should be refrigerated to reduce volatilization.
4. Remove stopper and let stand for two minutes.
5. Centrifuge for one minute at 1600 r. p. m. Four layers will appear in the tube: (a) an ether layer on top, (b) a plug of fecal detritus, (c) an M.I.F. layer, (d) the sediment containing protozoa and helminth eggs on the bottom.
6. Loosen the fecal plug by ringing with an applicator stick.

TABLE 1

Comparative findings of protozoa by technics 1 and 6†*

Species	Diagnosed by Technic No.				
	1 plus 6 (total)	1		6	
		No.	%	No.	%
E. histolytica (large race) trophozoites	11	5	45	10	91
E. histolytica (small race) trophozoites	19	13	68	12	63
E. coli trophozoites	32	17	53	22	69
E. nana trophozoites	26	12	46	21	81
I. bütschlii trophozoites	6	5	83	4	67
G. lamblia trophozoites	1	1	100	1	100
C. mesnili trophozoites	8	5	62	5	62
E. hominis trophozoites	4	—	—	4	100
T. hominis trophozoites	2	2	100	1	50
D. fragilis trophozoites	1	1	100	1	100
Total trophozoites	110	61	55	81	74
E. histolytica (large race) cyst	34	17	50	30	88
E. histolytica (small race) cyst	37	22	59	32	86
E. coli cyst	63	37	59	63	100
E. nana cyst	55	33	60	52	94
I. bütschlii cyst	17	9	53	16	94
G. lamblia cyst	12	9	75	9	75
C. mesnili cyst	2	1	50	2	100
E. hominis cyst	6	2	33	5	83
Total cyst	226	130	58	209	92
Total all protozoa	336	191	57	290	86

* (1) MIF, Vial preservative stain technic.
† (6) MIF, ether concentration technic.

TABLE 2

Combination of technics to increase the diagnostic yield for protozoa

	Comb. Total Identified by Both 1 and 6	Identical Recovery by Technics 1 and 6	No. Identified by Technics	
			1 only	6 only
E. histolytica (large race) trophozoites	11	4	1	6
E. histolytica (small race) trophozoites	19	6	7	6
E. coli trophozoites	32	7	10	15
E. nana trophozoites	26	7	5	14
I. bütschlii trophozoites	6	3	2	1
G. lamblia trophozoites	1	1	—	—
C. mesnili trophozoites	8	2	3	3
E. hominis trophozoites	4	—	—	4
T. hominis trophozoites	2	1	1	—
D. fragilis trophozoites	1	1	—	—
Total trophozoites	110	32	29	49
E. histolytica (large race) cyst	34	13	4	17
E. histolytica (small race) cyst	37	17	5	15
E. coli cyst	63	37	—	26
E. nana cyst	55	30	3	22
I. bütschlii cyst	17	8	1	8
G. lamblia cyst	12	6	3	3
C. mesnili cyst	2	1	—	1
E. hominis cyst	6	1	1	4
Total cyst	226	113	17	96
Total all protozoa	336	145	46	145

(1) MIF, Vial preservative stain technic.
(6) MIF, ether concentration technic.

7. Quickly, but carefully, pour off all but the bottom layer of sediment.

8. Thoroughly mix this sediment, pour a drop on a slide, mount with a cover glass, and examine.

The time required to prepare the M.I.F.C. specimen for examination is about four minutes.

RESULTS

All 100 fecal specimens were examined by technics 1 through 6. Technics 1 and 6 were compared for protozoa recovery and findings are summarized in Table 1. The results show that both trophozoite and cyst forms of protozoa are concentrated by technic 6. The diagnostic yield for each species of protozoa was increased by use of the M.I.F.C. method.

Table 2 shows the increase in diagnostic yield obtained when a combination of technics 1 and 6 are employed. In 46 instances, protozoa cysts or trophozoites demonstrated by technic 1 were not found by 6. On the other hand, 145 protozoa forms demonstrated by technic 6 were not identified by 1.

For efficiency in demonstrating helminth eggs, technics 2, 3, 4, 5 and 6 were

TABLE 3

Comparative findings of helminths by technics 2, 3, 4, 5 and 6

Species	Total Number of Infections by Technics 2, 3, 4, 5 and 6	Diagnosed by Technic No.									
		2		3		4		5		6	
		No.	%	No.	%	No.	%	No.	%	No.	%
A. lumbricoides	53	29	55	28	53	38	72	27	51	52	98
H. nana	13	9	69	7	54	7	54	6	46	9	69
Hookworm	8	5	63	2	25	1	13	3	38	8	100
Trichostrongylus	33	16	49	5	15	7	21	12	36	20	61
H. diminuta	1	—	—	—	—	—	—	—	—	1	100
E. vermicularis	9	5	56	3	33	1	11	2	22	6	67
M. javanica	1	—	—	—	—	—	—	1	100	1	100
S. mansoni	53	3	6	2	4	24	45	20	38	52	98
S. haematobium	12	—	—	—	—	3	25	1	8	9	75
Total	183	67	37	47	26	81	44	73	40	158	86

(2) Brine flotation technic. (3) Zinc sulfate flotation technic. (4) Formalin ether sedimentation technic. (5) Acid ether sedimentation technic. (6) M.I.F.C. technic.

TABLE 4

Diagnostic. Results obtained by a combination of technics

Species	Total Number of Infections by Technics 2, 3, 4, 5 and 6	Diagnosed by Combined Technics									
		2 and 4		2 and 5		3 and 4		3 and 5		6	
		No.	%	No.	%	No.	%	No.	%	No.	%
A. lumbricoides	53	42	81	35	67	41	79	36	69	52	98
H. nana	13	10	77	10	77	10	77	9	69	9	69
Hookworm	8	5	63	6	75	3	38	4	50	8	100
Trichostrongylus	33	20	61	25	76	11	33	14	43	20	61
H. diminuta	1	—	—	—	—	—	—	—	—	1	100
E. vermicularis	9	6	67	6	67	4	44	4	44	6	67
M. javanica	1	—	—	1	100	—	—	1	100	1	100
S. mansoni	53	25	47	22	41	25	47	21	40	52	98
S. haematobium	12	3	25	1	8	3	25	1	8	9	75
Total	183	111	61	106	58	97	53	90	49	158	86

(2) Brine flotation technic. (3) Zinc sulfate technic. (4) Formalin ether sedimentation technic. (5) Acid ether sedimentation technic. (6) M.I.F.C. technic.

compared. These findings are summarized in Table 3. The M.I.F.C. method increased the diagnostic yield for each species of helminth eggs. The recovery of helminth eggs by the use of a combination of technics is illustrated in Table 4. As shown in this table, the M.I.F.C. method recovered a greater number of eggs for each species of helminth than any combination of the other technics tested.

DISCUSSION

This laboratory had previously compared technic 1 with the most common methods used in the diagnostic laboratory for protozoa, i.e., the direct film saline method, unstained and iodine stained, plus the iron hematoxylin stained permanent mount, and found that with technic 1 the recovery of all species of protozoan cysts and trophozoites was equal to or greater than the recovery obtained by a combination of these standard methods. Subsequently, technics 1 and 6 have been compared for protozoan recovery. The 46 instances in which protozoan forms were diagnosed by technic 1 and not by 6 indicate that some of the protozoa may have been removed by straining, but the higher diagnostic yield obtained by technic 6 shows that concentration, and the greater visibility obtained by the removal of particulate material in the feces, rendered diagnosis of both cysts and trophozoites forms of protozoa easier, and thus more than compensated for the loss.

It was observed that the number of helminth eggs recovered by each technic was directly proportional to the specific gravity of the solutions employed. Brine solution had a specific gravity of 1.200 and floated a higher percentage of eggs than zinc sulfate solution, which has a specific gravity of 1.180. The opposite is true for sedimentation-centrifugation technics. The acid ether (specific gravity 1.028) recovered fewer eggs than formalin ether (specific gravity 1.012) and the M.I.F.C. (specific gravity 0.983) recovered more eggs than either sedimentation method. The specific gravity of the M.I.F. solution cannot be decreased and still float the fecal detritus with ether. In rare cases, when the M.I.F. preserved specimen is improperly prepared, it may be necessary to add a few drops of tapwater to the M.I.F. mixture if the ether will not mix with the preserved specimen after shaking. This is done to increase the specific gravity of the M.I.F. solution.

In many laboratories, two concentration technics are employed, a flotation type for non-operculated eggs or for eggs light enough to float in the solution used, and a sedimentation type for all other eggs. Recovery by the M.I.F.C. method indicates there is no need for two examinations since the number of eggs diagnosed by this technic is greater than that obtained by any combination of the other methods tested. This is demonstrated in Table 4.

One reason for the great variation in number of helminth eggs recovered in flotation type and in sedimentation type methods is the fact that there were 65 cases of *Schistosoma* diagnosed by all methods. The flotation methods did not concentrate many of these eggs. Only three cases were diagnosed by the brine technic and two by the zinc sulfate method. In each case, the eggs were lodged in a patch of mucus, and except for this fact it is unlikely that they would have been diagnosed.

The M.I.F.C. technic revealed as many of each species of helminth eggs as any other method and in many instances the recovery was about double, as in the case of *Ascaris lumbricoides*, *Schistosoma mansoni* and *Schistosoma haematobium* (Table 3).

The results of this evaluation study indicate the superiority of the M.I.F.C.

technic for the recovery of helminth ova. By the addition of ether to the M.I.F. solution most of the detritus was removed, leaving a clear and stained preparation to be examined. At the same time, the ether had no apparent effect on the stain. Centrifugation accelerates sedimentation, permitting examination of the preparation a few minutes after the stool is received in the laboratory.

Nine species of helminth eggs were diagnosed in this study. These helminths are all pathogenic to man with the exception of the nematode, *Meloidogne*, which infests vegetables and is at times recovered from human stools. No infection with *Trichuris trichiura* or *Heterophyes* were found in this study although both species have been demonstrated by the routine use of the M.I.F.C. technic in this laboratory. It is felt that since eleven different species were diagnosed with this technic, it is satisfactory for most all species of helminth eggs.

SUMMARY AND CONCLUSIONS

A modification of the M.I.F. (merthiolate-iodine-formaldehyde) technic (Sapero and Lawless, 1953) for identification of stool parasites is described. This technic, utilizing the principle of sedimentation, and referred to as the M.I.F.C. (merthiolate-iodine-formaldehyde-concentration) technic, showed improved diagnostic accuracy for stool protozoa and helminth eggs.

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LEPTOSPIROSIS OF MAN AND ANIMALS IN URBAN, RURAL AND JUNGLE AREAS OF SOUTHEAST ASIA

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Interest in leptospirosis has been renewed in Malaya with the appearance of clinical disease among members of the security forces operating on patrol in the primary jungle (Broom, 1953; Hughes, in discussion following Broom's paper; Ley *et al.*, to be published). Fletcher (1928) had presented unequivocal evidence for the occurrence of leptospirosis in Malaya and, more recently, Danaraj (1950) reported 19 human cases from Singapore. This report is concerned with the prevalence in Malaya of leptospirosis in members of the civil population, domestic animals and wild rodents as determined by serologic and cultural methods. It also deals with serologic studies on members of the indigenous population of British North Borneo and with a limited number of cultures from rodents of North Borneo, Thailand and Korea.

The present studies indicate that leptospiral infections are indeed common among man and animals in Malaya and North Borneo and are also present in certain rodents of Thailand and Korea.

MATERIALS AND METHODS

Selection and collection of sera for serologic survey. In Malaya sera were collected in 1951 from persons of three ethnic groups. These three groups were selected because they differ widely in many of their habits and customs, in their contact with the forest, and in their participation in agricultural activities, thereby providing a wide range of possibilities of contact with contaminated water, forest and town rodents and domestic animals.

(1) *Village Malays.* Sera were obtained from 29 Malays (28 males, 1 female) who resided in villages in the general vicinity of Kuala Lumpur, Malaya. Some of the samples came from volunteers who were being employed at the time in the studies on scrub typhus (Smadel *et al.*, 1952). Malays from this vicinity may be considered as partly urbanized but most continue to have some contact with agricultural activities.

(2) *Urban Chinese.* Practically all of the 25 Chinese who provided sera were inhabitants of Kuala Lumpur; 13 were males and 12 females. Many were patients with diverse ailments on the wards of the General Hospital.

(3) *Sakai.* Eight serum specimens were obtained from members of the forest-dwelling aborigines of Malaya commonly called by the collective term, the Sakai (Williams-Hunt, 1952). The bleedings were made at a government sponsored village about 10 miles from Kuala Lumpur where Sakai of different racial origins and from different parts of Malaya were sheltered. The forest people are very shy and it was only with the help of the late Major Peter Williams-Hunt, who

had gained their confidence through his studies and work in the capacity of Adviser on Aborigines for Malaya, that these few specimens were obtained. The Sakai hunt for food within the primary forest but also practice a limited amount of shifting agriculture. Their contact with wild rodents and with forest streams and swamps may be considered intimate.

In addition to the groups just mentioned, 27 sera were collected in 1951 from abattoir workers, butchers and tanners, living in different parts of Malaya. This occupational group was comprised of Indians, Malays and Chinese.

The serum specimens from British North Borneo were obtained in the West Coast Residency by Doctor J. R. Audy in 1952 from members of the indigenous population who were serving as porters and volunteers in studies on leech repellents undertaken by a joint United States Army-Colonial Office Medical Research Unit. These persons were primarily adolescent and young adult males of somewhat mixed origin, although the majority belong to the tribes known as the Dusuns. Persons in this area are intimately associated with the forest, its streams and wild rodents. Some cultivate their rice in flooded fields while others employ the dry method.

Serum specimens from domestic animals were obtained through the cooperation of the Department of Veterinary Services of Malaya and came from widely separated localities. No attempt is made to classify them according to place of collection. The canine specimens, with the exception of one from Johore Baru, were all obtained in Kuala Lumpur. Horses indigenous to Malaya are rare and the great majority of those tested had been imported into the country from Australia, Indonesia, India and Japan. Furthermore, since about half were race horses, they had been transported widely throughout Malaya from one race track to another. Many of the cattle had been imported also.

The serum specimens taken from Malayan rodents were obtained from animals trapped in the city of Kuala Lumpur and in sundry forest and scrub areas nearby. Identification and bleeding of rodents were performed in the laboratories of the Institute for Medical Research.

Serologic tests. All serum specimens were stored in the frozen state in Malaya until they were transported with wet ice by air to the Army Medical Service Graduate School in Washington, D. C. Here they were again frozen and stored at −20°C. pending their use in the tests.

The agglutination-lysis test (Gochenour *et al.*, 1953; van Thiel, 1948; Gsell, 1952; Gochenour and Yager, 1952) using at least 16 different leptospiral strains[1]

[1] The leptospiral strains employed as antigens in the agglutination lysis test in all instances were as follows: *L. andaman*; *L. semeranga*; *L. djatzi* (*bataviae* serogroup); *L. bataviae* van Tienen; *L. pomona*; *L. djasiman*; *L. sentot*; *L. autumnalis* Akyiami A; *L. ballum*; *L. canicola*; *L. icterohemorrhagiae* AB; *L. pyrogenes Salinem*; *L. alexi* (*pyrogenes* serogroup); *L. grippotyphosa* Moscow V; *L. borincano* (*hebdomadis* serogroup); *L. sejroe*. In addition, all of the sera from North Borneo were tested against *L. javanica* and some, but not all, of the animal sera from Malaya were tested against *L. autumnalis* Fort Bragg, *L. javanica*, and *L. schüffneri* (vleermus 90c). This group of strains was selected on the basis of previous serologic classification studies by the Veterinary Division, Army Medical Service Graduate School (Gochenour and Yager, 1952).

was the test employed to detect leptospiral antibodies. Only antibody titers of 1:100 or greater were considered significant. When a given serum reacted with several antigens, interpretation took into consideration the known cross reactions and only the minimal number of groups of leptospiras which could account for the observed antibody pattern was listed in the results. Reactions are recorded according to serogroup only.

Selection and collection of materials for leptospiral isolations. Specimens for leptospiral isolations from human beings were taken from patients suspected of having the disease in the Military Hospital, Kinrara, near Kuala Lumpur. Epidemiologic considerations of these military cases will appear in another publication (Ley *et al.*, to be published).

In Malaya, rats, squirrels and shrews were trapped in various habitats in the vicinity of Kuala Lumpur. They were brought to the laboratories of the Institute for Medical Research in separate cages where they were identified before the organs were cultured.

The rodents from Thailand were collected by Doctors Mali Thaineua and Robert E. Elbel in the regions of Ratburi, Banpong, and Kanachanaburi, Ladya, in connection with plague studies by the Thai Ministry of Public Health, Division of Communicable Diseases and the United States Special Technical and Economic Mission to Thailand. They were shipped by air in individual cages to the Army Medical Service Graduate School in Washington, D. C., where the isolation studies were performed. Care was taken to avoid cross infection by keeping the animals as widely separated as possible under the conditions of transport.

The two specimens of *Rattus whiteheadi* from North Borneo were trapped near Tenompak (elev. 4500 ft.) by members of a joint United States Army-Colonial Office Medical Research Team operating there in September 1953 and were also transported to the United States by air. The Korean rodents were trapped near Yonch'on by members of the Field Unit of the Commission on Hemorrhagic Fever of the Armed Forces Epidemiological Board and were likewise transported to the United States for study.

Isolation methods. Fletcher's medium (Fletcher, 1928) was used for the isolation of leptospiras from all sources. Defibrinated blood was the inoculum from suspected human leptospiral infections while material for culture from the rats, squirrels and shrews was taken from a single kidney or from a pool of kidney, liver and spleen ground in saline with a Ten Broeck grinder to form a uniform homogenate. The cultures were incubated at room temperature in the dark, and examined periodically for leptospiras under the dark field microscope. On occasion, such cultures were freed of bacterial contaminants by inoculation into young guinea pigs or hamsters followed by subculture in Fletcher's medium of the heart blood obtained on the fourth or sixth day (Gochenour *et al.*, 1953; van Thiel, 1948; Gsell, 1952). All the strains isolated were assembled together at the Army Medical Service Graduate School for final identification.

Identification of the leptospiral strains. The leptospiral strains isolated were identified by cross agglutination-lysis studies performed by the Department of Veterinary Bacteriology at the Army Medical Service Graduate School (Goche-

nour and Yager, 1952). From each group of strains which behaved in a uniform manner, a single strain was selected for cross absorption studies. The detailed characterization studies on these strains will appear in another publication (Alexander *et al.*, to be published).

RESULTS

I. Serologic surveys

Interpretation of the results of the serologic surveys for leptospiral infections requires special comment. While it is reasonable to assume that a positive reaction in the leptospiral agglutination-lysis test indicates previous leptospiral infection, the identification of the infecting strain by examination of the serum at unknown intervals after infection is subject to certain limitations. Among the factors which may affect the specificity of the test are the following: (1) variable degrees of cross reactions occur among different leptospiras; (2) the strains employed as antigens may not be identical to the strains causing infection in a given area; and (3) repeated infection with different strains or simultaneous infection with multiple strains may alter the pattern of serologic response. All of these factors are of potential importance in Malaya (and Borneo) where multiple strains exist and individual persons may be exposed to different strains at various times in their lives. Despite such limitations, the serologic results strongly suggest that a multiplicity of strains of leptospiras infect men and animals in the areas under investigation. This is in accord with the results of isolation tests which are discussed elsewhere in this report.

Survey for leptospiral antibodies of human civil population groups in Malaya and North Borneo. In both Malaya and North Borneo, about one-fourth of the people tested were found to possess antibodies which reacted with members of one or more serologic groups of *Leptospira*. Table 1 summarizes the data obtained from 89 Malayan and 54 Bornese sera. In the Malayan series there was little difference in the proportion of positive reactions between the Village Malays and Urban Chinese. Furthermore, the abattoir workers and butchers did not exhibit a higher incidence of positive reactions than did the Malay or Chinese groups selected without regard to specific occupation. Only eight Sakai sera were available for testing but half of these contained leptospiral antibodies which is consistent with the idea that these people may live at greater risk of infection.

Survey for leptospiral antibodies in domestic animals of Malaya. The search for leptospiral antibodies in the various domestic animals from diverse parts of Malaya, presented in summary form in Table 2, revealed that the incidence of positive reactions in the groups tested varied widely, i.e., from one of seven buffalos to 19 of 29 horses. Despite the fact that some of these animals, e.g., horses and some cattle, had their origin outside Malaya, the generally high incidence of leptospiral antibodies among these and the indigenous animals also points to a widespread experience of domestic animals in Malaya with leptospiras. Furthermore, the high incidence of positive reactions in dogs recalls Fletcher's (1928) demonstration of leptospiral infections in these animals in 1928. The significance of these findings is enhanced by the recent emphasis on the role of leptospiral

infections of domestic animals in the epidemiology of the disease in man (Larson, 1953; Gsell, 1953; Gochenour, 1952).

Survey for leptospiral antibodies in Malayan rodents. Rodents have long been recognized as important reservoirs of leptospiras in nature (van Thiel, 1948; Gsell, 1952). It is not surprising, therefore, that in the small series of Malayan rodent sera tested, a number of positive reactions was found (Table 3). Of

TABLE 1

Survey for leptospiral antibodies in different human civil population groups of Malaya and British North Borneo

Ethnic or Occupational Group	Age Group (Years)	Number Tested	Number Positive	Multiple Reactors	Antigens Giving Positive Reactions*
		Malaya			
I. Village Malays	-5	0	0	0	—
	6-10	1	0	0	—
	11-20	8	2	1	2, 5, 6
	21-30	14	2	1	1, 3, 5
	31-	6	1	0	3
		—	—	-	
		29	5	2	1, 2, 3, 5, 6
II. Urban Chinese	-5	1	0	0	—
	6-10	0	0	0	—
	11-20	14	3	1	3, 4, 1
	21-30	4	1	0	1
	31-	6	1	1	3, 9
		—	—	-	
		25	5	2	3, 1, 4, 9
III. Sakai	-5	0	0	0	—
	6-10	0	0	0	—
	11-20	2	2	2	1, 3, 6, 9
	21-30	5	1	0	6
	31-	1	1	0	1
		—	—	-	
		8	4	2	1, 6, 3, 9
IV. Abattoir Workers, butchers, tanners	Adults†	27	7	3	7, 3, 4, 1, 6
		British North Borneo			
V.	Indigenous adults‡	54	14	3	2, 3, 5, 6, 1, 8, 6

* Code numbers to leptospiral antigens are listed in order of frequency and refer to the following serotypes: 1, hebdomadis; 2, autumnalis AB; 3, pyrogenes salinem; 4, canicola; 5, icterohemorrhagiae AB; 6, bataviae; 7, grippotyphosa; 8, semeranga; 9, pomona; 10, djasiman; 11, ballum; 12, schüffneri; 13, sentot.

† Includes Malays, Chinese and Indians (all males).

‡ Mixed ethnic origins but mostly young adult Dusuns (all males).

TABLE 2

Survey for leptospiral antibodies in Malayan domestic animals

Animal	Number Tested	Number Positive	Multiple Reactors	Antigens Giving Positive Reaction*
Goats	13	2	0	2, 4
Sheep	5	1	0	1
Pigs	5	3	0	2, 9, 13
Horses	29	19	7	1, 4, 2, 8, 9, 3, 5, 6, 10, 11
Cattle	86	16	3	1, 12, 3, 2, 4, 8, 9
Oxen	12	5	2	1, 9
Buffalo	7	1	0	1
Dogs	19	8	1	4, 8, 1, 7, 10

* Code numbers to leptospiral antigens are listed in order of frequency and refer to the following serotypes: 1, hebdomadis; 2, autumnalis AB; 3, pyrogenes salinem; 4, canicola; 5, icterohemorrhagiae AB; 6, bataviae; 7, grippotyphosa; 8, semeranga; 9, pomona; 10, djasiman; 11, ballum; 12, schüffneri; 13, sentot.

TABLE 3

Survey for leptospiral antibodies in Malayan rodents

Rodent species	Number Tested	Number Positive	Multiple Reactors	Antigens Giving Positive Reaction*
Town				
Rattus rattus diardii	16	1	0	5
Forest				
Rattus whiteheadi	2	0	0	—
Rattus mülleri	4	3	0	1, 6, 7
Rattus rajah	2	0	0	—
Rattus sabanus	1	0	0	—
Totals	25	4	0	1, 5, 6, 7

* Code numbers refer to antigens of following leptospiral serotypes: 1, hebdomadis; 5, icterohemorrhagiae AB; 6, bataviae; 7, grippotyphosa.

particular interest is the low incidence of positive reactions in the town rat, *Rattus rattus diardii*. In contrast, three positive reactions were found in the four sera from *Rattus mülleri*, an animal which frequents the forest streams and is known as "water rat" to the Sakai (Harrison, 1949; Harrison and Traub, 1950).

II. Leptospiral isolations

Leptospiras isolated from man in Malaya. The human cases suspected of having leptospirosis were all members of military groups who had been on patrol in the jungle where presumably they acquired their infection. Eight serotype strains representing four different serologic groups of leptospiras were isolated and are listed in Table 4. Four belonged to the *pyrogenes* serogroup, two to the *hebdomadis*

TABLE 4

Summary of leptospiral isolations from man and animals in North Borneo and Malaya

Host	Number Cultured	Number Positive	Leptospiral Strains Isolated (Serogroup)
British North Borneo			
Rattus whiteheadi	2	1	icterohemorrhagiae
Malaya			
Man	32	8	pyrogenes (x4) hebdomadis (x2) icterohemorrhagiae (x1) sp.* (x1)
Rattus mülleri	17	3	hebdomadis (x2) grippotyphosa (x1)
Rattus rajah	10	1	schüffneri
Rattus sabanus	13	1	hebdomadis (x1)
Rattus cremoriventer	1	0	
Rattus bowseri	2	0	
Callosciurus tenuis	2	0	
Callosciurus notatus	3	0	
Callosciurus caniceps	1	0	
Tupaia glis	1	0	

* Strain of new serotype.

serogroup one member of the icterohemorrhagiae serogroup and one which gave serologic patterns differing from any strain known to us.

Leptospiras isolated from rodents: Since attention had been directed by the military cases to the forest and scrub areas as important potential sources of infection, efforts were concentrated on culturing the small mammals of these regions for leptospiras. Indeed, three species of Malayan rodents were found positive for the cultural methods (1) *Rattus mülleri*, found along streams in the primary forest; (2) *Rattus rajah*, which inhabits the primary forest and jungle edge; and (3) *Rattus sabanus*, which is found only in the primary jungle (Harrison, 1949; Harrison and Traub, 1950). Of the other rats, squirrels and shrews, the number examined was small and the negative results do not necessarily exclude these groups from consideration as potential carriers. Nevertheless, it is of some interest from the epidemiological standpoint that the only forest rodents found to be positive for leptospiras by culture or by serologic methods were those whose usual habitat was on the ground or near water. The strains isolated from the Malayan rodents belonged to the *hebdomadis*, *grippotyphosa* and *schüffneri* serogroups.

The single strain isolated in North Borneo was a member of the *icterohemorrhagiae* serogroup from *Rattus whiteheadi*, another forest inhabitant.

Of the five kinds of animals from Thailand which were cultured, only species of *Bandicota* yielded positive results. *Bandicota bengalensis*, a large rodent native to Northern India, is also found in Burma where Taylor and Goyle (1931) re-

TABLE 5

Summary of leptospiral isolations from rodents of Thailand and Korea

Host	Number Cultured	Number Positive	Leptospira Strains Isolated (Serogroup)
Thailand			
Rattus rajah	6	0	
Rattus rattus thai	8	0	
Bandicota bengalensis	39	5	autumnalis (x5)
Bandicota sp.	1	1	autumnalis
Menetes berdmorei	5	0	
Korea			
Microtus fortis	3	0	
Cricetulus triton	5	0	
Mus musculus molossinus	3	1	icterohemorrhagiae
Apodemus agrarius	9	1	icterohemorrhagiae
Micromys minutus	2	0	

ported it naturally infected with leptospiras. It is a pest of grain crops and is said to invade houses readily (Harrison, 1949; Harrison and Traub, 1950). The leptospiral strains isolated from *B. bengalensis*, all members of the autumnalis serogroup, have wide distribution in Asia (van Thiel, 1948; Gsell, 1952). Little is known of leptospirosis in Thailand but a series of 53 cases was reported recently from Bangkok. Most of the strains implicated in these cases remain unidentified; however, *L. bataviae* and *L. rachmat* were thought to have been among them (Sundharagiati and Buspanovich, 1951).

The 22 Korean rodents tested were collected from only one area north of Seoul. The two species of rodent from which a member of the *icterohemorrhagiae* serogroup was isolated, i.e., *Apodemus agrarius* and *Mus musculus molossinus*, are essentially field inhabitants. The role of field mice of various kinds in the epidemiology of leptospirosis in different parts of the world is well known (van Thiel, 1948; Gsell, 1952).

DISCUSSION

The occurrence of leptospiral antibodies in about one-fourth of the human civil population, in about one-third of the domestic animals and in about one-sixth of all the wild rodents tested indicates that leptospirosis is a common infection in Malaya. These findings receive support from the isolation from men and rodents of leptospiras belonging to several serogroups.

In Malaya, the population is comprised predominantly of peoples of Malay and Chinese origin while numerically those of Indian and European origin constitute a relatively minor segment. The Malays tend to live together in villages in rural or surburban settings where their lives are governed largely by the customs and religious practices peculiar to their culture. Such Malays usually engage in agricultural practices regardless of other employment. The Chinese of Kuala Lumpur, on the other hand, tend to live a kind of urbanized existence

typical of many oriental cities; and their customs differ in many striking ways from those of the Malays. Therefore, it is of particular interest that the incidence of leptospiral antibodies among the Village Malays and Urban Chinese did not differ substantially from one another. It is of some interest that the incidence of positive reactors among abattoir workers and butchers was of the same order of magnitude as that found among the more randomly selected Malay and Chinese groups. Since, in other parts of the world this occupation has often been attended with an increased chance for contracting leptospirosis (Gochenour, 1952; Larson, 1953; Gsell, 1953), one might conjecture that the hazard imposed on this group even by the high incidence of leptospirosis in domestic animals in Malaya does not outweigh in importance that which obtains in the general environment.

Domestic animals are being recognized more and more as important sources of human leptospiral infections (Gochenour, 1952; Larson, 1953; Gsell, 1953). In Malaya, leptospiral antibodies were found in at least a portion of the specimens from each group of animals tested. The proportion was especially high among the horses, oxen, pigs and dogs.

The primary jungle, which still covers an estimated 70 per cent of Malaya and is to be distinguished from the scrub vegetation seems to deserve much more consideration as an endemic focus of leptospirosis than it has received. Certainly, the results of the serological studies in the small group of forest-dwelling aborigines are compatible with this idea. The exploratory search for reservoirs of leptospirosis in the jungle was well rewarded since three species of rodent not found outside the primary forest, or its edge, yielded positive evidence by both serologic and cultural methods.

Thus, Fletcher's (1928) findings in rodents[2] from rural areas, the occurrence of outbreaks of leptospirosis in jungle patrols (Hughes, in discussion following Broom's (1953) paper; Ley *et al.*, to be published) and the current studies demonstrating infection in rodents from deep in the primary forest all point to both types of environment as constituting important endemic foci of leptospirosis in Malaya. Further support for this is gained from Fletcher's (1928) isolation of leptospiras from waters of impounded jungle streams and by the fact that swimming in some such pools has been followed by febrile illnesses diagnosed on clinical grounds as probable leptospirosis. It is not altogether surprising that leptospirosis should be prevalent in an area such as this with its warm climate, heavy rainfall, numerous streams and abundance in numbers and varieties of rodents.

Evidence at hand points to the existence of a multitude of different kinds of leptospiras in Malaya. Indeed, representatives of six different serogroups were

[2] The exact identity of the rats mentioned by Fletcher is not entirely clear and his statements regarding them were somewhat ambiguous. However, Harrison (Harrison, 1949; Harrison and Traub, 1950), in a discussion of this matter, suggests that the town rat to which he referred as *Mus rattus griseiventer* is in all probability *Rattus rattus diardii* and the rats from the country districts which he calls "? *Mus rattus diardii*" are most likely *Rattus rattus argentiventer*. The rats Fletcher caught on an oil palm estate were probably mainly *Rattus rattus jalorensis*, but *R. r. diardii* and *R. r. argentiventer* may also have been represented.

TABLE 6

*Summary of leptospiras isolated in Malaya**

Present Study		Fletcher (1928) Human Isolates	Broom (1953) Human Isolates
Rodent isolates	Human isolates		
Serogroup			
Hebdomadis	pyrogenes	pyrogenes	bataviae
Grippotyphosa	hebdomadis	autumnalis	canicola
Schüffneri	icterohemorrhagiae	icterohemorrhagiae	autumnalis
	sp. (new serotype)	3 unidentified strains	

* Listed in order of frequency observed.

actually isolated in the present study. When these are considered along with the findings of Fletcher (1928) and Broom (1953), the number of leptospiras identified by characterization of isolated strains is brought to a total of eight known serogroups and four types which remain unidentified with known groups. These are listed in Table 6. Furthermore, the serologic studies lead one to suspect that there are a number of strains present in Malaya in addition to those already isolated, a reasonable assumption in view of the fact that at least 18 different strains have been identified in nearby Indonesia (Collier, 1948).

On the basis of serologic studies, leptospirosis would appear to be prevalent in British North Borneo just as it is in Malaya and in Indonesia (Collier, 1948). Again, the evidence on serologic grounds points to the occurrence of multiple strains. The single isolation of a member of the *icterohemorrhagiae* serogroup from a jungle rat suggests that here, too, the forest rodents may be carriers. Previous studies of leptospirosis in Borneo appear to have been confined to South Borneo where Lingen (1933) and Bessem (1936) each reported human infections following swimming or patrol activities with exposure to water.

The isolations from field rodents of Thailand and Korea are included here since they serve the purpose of recording unequivocally the presence of leptospiras in carefully identified hosts from regions of Asia where definitive information on these matters is limited.

The present study has been one of an exploratory nature and, as such, is subject to certain limitations. Neverless, it provides a number of basic observations which may serve as guides for the planning of more detailed studies in the future. Although considerable, and deserved, emphasis has been placed on leptospirosis in the rural and forest areas in Malaya, the fact that the urban population was affected as well should not be ignored. Indeed, the open sewers and streams, market places and shops, and many rodents, dogs, and oxen probably provide ample opportunity for contact with leptospiras.

SUMMARY

An exploratory, but systematic, serologic survey in Malaya indicated that leptospirosis is very prevalent there with about one-fourth of the human population, one-third of the domestic animals and about one-sixth of the wild rodents

tested exhibiting antibodies to one or more strains of leptospiras. In the human population, the incidence of antibodies was uniformly high in urban, rural and jungle inhabitants alike while, among the domestic animals, horses, oxen, pigs, and dogs exhibited particularly high incidences.

Although evidence points to both town and rural agricultural areas as frequent sources of infection, attention has been directed in particular to the primary jungle in Malaya as an important endemic focus of leptospirosis. Here, three species of rodent which inhabit the primary forest (*Rattus mülleri*, *R. rajah*, and *R. sabanus*) gave evidence of leptospiral infection by serologic or cultural methods. Both serologic and cultural findings point to the presence of a multiplicity of leptospiral strains in Malaya. Thus, leptospiral strains of six different serogroups (*hebdomadis*, *pyrogenes*, *icterohemorrhagiae*, *grippotyphosa*, *schüffneri* and one apparently new serotype strain) were isolated from man and wild rodents bringing the total number isolated here to date to eight known serogroups and four incompletely identified strains.

A more limited survey in British North Borneo demonstrated leptospiral antibodies in about one-fourth of the indigenous population and a strain of the *icterohemorrhagiae* serogroup on isolation from a jungle rat (*Rattus whiteheadi*). The findings suggest that here, too, leptospirosis is prevalent and is caused by a multiplicity of strains.

A number of wild rodents from Thailand and Korea were cultured for leptospiras. *Bandicota bengalensis* and *Bandicota sp.* from Thailand yielded strains of the *autumnalis* serogroup while both *Mus musculus molossinus* and *Apodemus agrarius* from Korea yielded strains of the *icterohemorrhagiae* serogroup.

ACKNOWLEDGMENT

The authors are grateful to Dr. J. W. Field, Director of the Institute for Medical Research in Kuala Lumpur, to members of the staff of the Institute, to the Director and Medical and Nursing Staffs of the British Military Hospital, Kinrara, Malaya, and to Doctors W. Neal and T. Abbott, Directors of Medical Services in British North Borneo, for their unreserved cooperation and assistance in this work.

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THE VALUE OF THE QUANTITATIVE COMPLEMENT-FIXATION TEST FOR SYPHILIS IN LEPROSY

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It has been reported by various workers from different countries that the frequency of positive reactions in serologic tests for evidence of syphilis remains high when the sera of leprous patients are examined despite the use of cardiolipin antigen. This has been true for the various flocculation tests and the Kolmer complement-fixation technic (Table 1). Results of this kind seem to be accepted as unavoidable despite the experience of Maltaner who, in 1940, examined the sera of 47 cases of leprosy by the quantitative complement-fixation technic and found that 7 of the 8 sera which reacted were from individuals with histories of "venereal exposure or promiscuity". The quantitative technic yielded equally discriminative information on the 60 sera from leprosy patients that were examined in the Washington Serologic Conference in 1942 (Parran *et al.*, 1942). Thus there has been suggestive evidence for a dozen years that the quantitative complement-fixation method has much to offer in the recognition of syphilitic infection among leprous individuals. The present study was undertaken to test the specificity of the quantitative technic using cardiolipin antigen. Maltaner's studies had been made before the discovery of cardiolipin antigen.

MATERIALS

1. Sera were collected from patients suffering from the lepromatous form of leprosy, since those yield a higher degree of false-positive reactions when tested for syphilis (Faget and Ross, 1944). Tuberculosis was ruled out in each of the patients examined in this series by extensive clinical examination in order to insure that positive results with the tubercle bacilli extract were due only to leprosy.

2. Cardiolipin antigen No. 72 (Pangborn *et al.*, 1951) was employed as recommended by Maltaner and Maltaner (1945) in maximally reactive doses for the detection of the syphilis reagin. Extract of tubercle bacilli was prepared according to the specifications of Pangborn (1952) and standardized by the Maltaners' technic with leprous sera (Almeida, to be published). The relationship of this antigen with leprous sera will be published elsewhere.

3. The syphilitic sera were diluted in normal human serum. All other reagents were diluted in isotonic borated saline, pH 7.6 (Wadsworth, 1947).

METHODS

The technic employed was that of Wadsworth, Maltaner and Maltaner (Wadsworth, 1947). The time and temperature allowed for fixation varied with

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TABLE 1

Comparison of results obtained by different investigators with cardiolipin and with crude antigens in serologic tests for syphilis with leprous sera

Author	Total Cases	Per Cent Positive Results Obtained with Leprous Sera					
		With crude antigens				With cardiolipin	
		Kahn	Kolmer	Mazzini	Meinicke	VDRL microflocculation	Kolmer
Trespalacios-Otero (1951)	312	47.7		53.8	15.0	54.5	
Utrilla (1947)	18	22.2			37.8	61.2	
Ross-Gemar (1949)	225	70.5	43			49.5	64
Portella-Almeida (1952)	152					27.0	
Rein-Bossak (1946)	37			62.0		51.5	
Shively-Kuhns (1950)	120	52.5				26.0	
Grasso (1951)	16	100				18.8	
Kvittingen *et al.* (1952)	169	37.9			9.5	21.9	
Kvittingen *et al.* (1952)	194	30.4			14.4	16.9	
Portnoy *et al.* (1952)	19	79	17.6	52.6		73.7	33

TABLE 2

Results in 467 lepromatous leprosy sera by the quantitative complement-fixation test, with cardiolipin and tubercle bacilli extract antigens

	Results with	
	Cardiolipin	Tubercle bacilli extract
Reaction	28 (6.0%)	413 (88.4%)
No reaction	439 (94.0%)	54 (11.6%)
Total	467	467

the system used, being 4 hours at 3°–6°C. for syphilis, and 1½ hours in a 37°C. water bath for leprosy. For both systems, 15 minutes in the 37°C. bath was allowed for hemolysis. All degrees of hemolysis were read on the supernatant fluids employing a photoelectric colorimeter, after diluting the reaction system with cold saline (Almeida, 1950). The reactions were evaluated in terms of the relative amounts of complement required for 50 per cent hemolysis under the different conditions of the test, that is, in the presence of serum or antigen alone, or in combination. The relative amounts of complement required under given conditions to give 50 per cent hemolysis can be read from previously prepared charts. The titers were calculated as the ratio of the number of units of complement required for 50 per cent hemolysis with serum plus antigen and the number of units of complement required for 50 per cent hemolysis with serum alone.

RESULTS

1. Four hundred sixty-seven cases (treated and nontreated) of lepromatous leprosy were examined with both the cardiolipin and the tubercle bacilli extract antigens, with the results shown in Table 2. Of the 467 lepromatous sera 413

(88.4 per cent) gave titers higher than 6 with the tubercle antigen (Table 2), and only 6.0 per cent (28 sera) reacted with cardiolipin.

2. Blood from 133 of the 439 leprous patients recorded in Table 2 who gave negative reactions in the quantitative complement-fixation test for syphilis were retested after three months or repeatedly. Samples from 67 patients were tested three times, 34 four times, 24 five times, and 8 were tested on six occasions at three-month intervals. The subsequent specimens were found to be negative in each instance.

3. One hundred eighteen of these 133 nonreacting specimens were also tested by other technics for the diagnosis of syphilis. Positive reactions were frequently encountered in conformity with the experience of others. The Kolmer complement-fixation technic with cardiolipin yielded 16.8 per cent that reacted; with Kolmer antigen, 12.5 per cent; the Kahn standard, 65 per cent; and the VDRL, 36 per cent.

4. Twenty-five of the 28 patients whose sera had reacted in the quantitative technic were found to have histories of venereal disease at some time in the past. Case histories were unobtainable from 3 patients.

The titers for syphilis of these 28 specimens using cardiolipin antigen were compared with the titers obtained for leprosy using the tubercle bacilli extract as test antigen. The results are shown in Table 3. There seems to be no relationship between the titers obtained by the two tests. In Table 4 the data for the five

TABLE 3

Comparison of degree of reactivity for syphilis obtained with specimens yielding various degrees of reactivity for leprosy

Complement-Fixation Reaction for Leprosy		Number of Cases Tested for Syphilis Giving Titers of			
Degree of reaction	Number of cases	No reaction	3–9.9	10–99.9	100 or greater
No reaction	54	53	0	1	0
3–9.9	39	28	6	5	0
10–99.9	262	251	8	3	0
100 or greater	112	107	0	3	2
Total cases.....	467	439	14	12	2

TABLE 4

Reactivity of sera from leprous patients with syphilis in the quantitative complement-fixation test using tubercle bacilli extract and cardiolipin as antigens

Patient	Titers in Quantitative Complement-Fixation Tests with	
	Tubercle bacilli extract	Cardiolipin
AGF	140	13
NI	143	121
LM	250	37
DSP	144	14
SFA	260	270

highest titers obtained with the tubercle antigen are arranged in comparison with the syphilitic titers.

5. All of the patients whose sera showed reaction in the quantitative complement-fixation test for syphilis were being given the regular sulfona treatment for leprosy. Further specific penicillin treatment for syphilis was instituted in those cases which yielded a positive reaction in the quantitative complement-fixation test for syphilis. Table 5 shows the effect of treatment on the titers determined for syphilis and for leprosy, and an apparent lack of correlation between the titer changes in the two tests. The serologic titers with cardiolipin decreased after penicillin treatment in the manner to be expected in nonleprous syphilitic patients.

TABLE 5

Effect of penicillin treatment upon the serologic curves in syphilis and leprosy, in patients treated regularly with sulfonas

Penicillin Treatment	No. of Cases	Change of Titers upon Treatment		
		Titer	In syphilis	In leprosy
Regular	13	increased	0	5
		decreased	10	2
		did not change	3	6
Irregular	3	increased	1	0
		decreased	2	0
		did not change	0	3
No treatment	2	increased	2	0
		decreased	0	2
		did not change	0	0

TABLE 6

Effect of specific treatments upon the serologic curves for leprosy and for syphilis†*

Date	Complement-Fixation Titers	
	Tubercle antigen	Cardiolipin
9/19/51	260	270
1/28/52	249	18
4/2/52	211	16
6/21/52	146	20
10/8/52	100	6
12/12/52	82	no reaction
2/11/53	110	no reaction

* Since September, 1951, patient received 582 ml. of promin and 6 million units of penicillin. The titers are presented both for cardiolipin and for tubercle bacilli extract.

† Case No. 154 (SFA), male, 32 years, white. Lepromatous leprosy. Five years ago had a syphilitic chancre, secondary stage after 6 months. Irregular treatment. Admitted to hospital in September, 1951, when the tests for leprosy and syphilis were performed.

In Table 6 are presented data illustrating one of the cases, in which penicillin treatment was instituted and the effect of this on the complement-fixation titers measured.

DISCUSSION

The evaluation of serologic procedures in terms of human specimens is commonly plagued by uncertainties regarding the true status of the patients. This is true of our cases quite as much as of many others. Despite this limitation it appears highly suggestive that only 6.0 per cent of the sera from our leprosy patients reacted in the quantitative complement-fixation test since the experience of others has consistently indicated a greater frequency of reaction. It is interesting to note that 88.4 per cent of these same sera reacted when tubercle antigen was used and that there was no evident relationship between the results in individual sera by the two tests as well as in the series as a whole.

The present series indicates that the quantitative technic provides a reliable criterion of syphilitic infection even in individuals concurrently leprous. This is indicated by the association of positive reactions with histories of syphilitic infection and the response of the titers in the quantitative test during treatment of the syphilis.

While it seems unnecessary to point out that the specificity of a serologic test depends on principle and procedure as well as the reagents used, it may be properly noted that if the present results are representative, the diagnosis of syphilis by laboratory procedures is feasible among individuals suffering from leprosy as well as others and that the quantitative complement-fixation test has unique value under such circumstances.

CONCLUSIONS

The quantitative complement-fixation test for syphilis was found to be highly specific as judged by all available criteria in the examination of 467 sera from patients with leprosy.

ACKNOWLEDGMENT

The cardiolipin and tubercle antigens were furnished through the courtesy of the New York State Department of Health, Division of Laboratories and Research, Albany.

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FILARIASIS IN LIBERIA[1]

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During the fall of 1951 a survey was made to determine the incidence of malaria in two small native villages located near Robertsfield, Liberia. It was observed that 7.3 per cent of the individuals examined in this preliminary survey had demonstrable microfilariae in day blood smears. This was in such marked contrast with the previous findings of Poindexter (1950) and Young (1953), who had both reported prevalence rates of less than one per cent at Robertsfield, that additional surveys and investigations were carried out during the following two years.

METHODS

Thick blood smears and/or scarification smears were made on nearly 2000 African inhabitants of Liberia. These examinations were done on residents of

a. Marshall Territory near the Liberian Institute of the American Foundation for Tropical Medicine and Robertsfield.
b. Divisions 21 and 22 of Firestone Plantations Co., Harbel.
c. Monrovia and Claratown, a suburb of Monrovia.
d. The Liberia Mining Co. at Bomi Hills.
e. The Lutheran Mission at Zorzor.
f. The Methodist Mission at Ganta.
g. The Rural Health Clinic at Gbanga.
h. Various towns in the southeast portion of the Central Province near the St. John River.

Nearly all of those examined were adults except that only school children were examined in the city of Monrovia. Arrangements for conducting the surveys were made with the chiefs or other government officials or members of the staff of the various organizations. Where this was not possible, the survey was conducted during a movie, party or other function which would induce the local population to present themselves for examination.

Thick blood smears made during the preliminary surveys at Monrovia and the Liberian Institute were prepared from a drop of finger blood spread to an area of approximately 5 x 15 mm. and stained with Giemsa. Subsequent thick blood smears were made using a larger quantity of blood so that the blood film would measure approximately 15 mm. square which was the same size as the blood film prepared by the scarification technique. These were stained by Delafield's hematoxylin.

[1] This work was done as part of the United States Technical Cooperation Administration Program in Liberia and was a collaborative project with the National Public Health Service of Liberia. Laboratory facilities were made available by the Liberian Institute of the American Foundation for Tropical Medicine.

The slides from the Monrovia school children were examined at the Laboratory of Tropical Diseases at Milledgeville, Ga. All the remaining slides were examined by African technicians at the Liberian Institute. All positives and at least 20 per cent of all slides reported negative were verified by the medical officer.

OBSERVATIONS

Many of the examinations for microfilariae were done in conjunction with surveys for onchocerciasis utilizing a scarification smear technique. In order to evaluate any difference in effectiveness between this type of examination and the more usual thick blood smear, 149 patients were examined simultaneously by both methods. Comparing the results obtained with using the thick blood smear with the scarification procedure, as shown in Table 1, there was very little difference noted in the number of patients demonstrated to have microfilariae of *Wuchereria bancrofti* and in the number of microfilariae found.

In order to ascertain the optimum time for making blood smears for the demonstration of microfilariae of *Wuchereria bancrofti*, hourly thick blood smears using 20 cmm. of blood were made on twelve subjects with filariasis for twenty-five hours. One patient had very high numbers of microfilaria. His results are reported separately while the other eleven are averaged together (Table 2).

All had an abrupt increase in numbers of microfilaria in the peripheral blood between 8 and 9 p.m. which fell rapidly but not as abruptly starting at 6 a.m. From a practical viewpoint, it was decided to make night blood examinations at 9 p.m. or as close to that time as possible.

In the above experiment, the average number of circulating microfilariae found during the time when night smears were usually made (i.e., 9 to 11 p.m.) was about 6 times that occurring between the hours of 8 to 10 a.m. when the usual clinic laboratory examinations were made. In order to compare the findings in actual practice with those determined experimentally, the records of 29 patients being treated for filariasis were evaluated. There were 14.5 times as many microfilaria in the night as in the day smears on routine clinic examination (Table 3).

A total of 1,395 African natives were examined in Liberia for microfilaria during the day and 520 at night. Forty-two or 3.0 per cent of the day smears and 53 or 10.2 per cent of the night smears contained microfilaria of *W. ban-*

TABLE 1

Comparison of results obtained by thick and scarification smear techniques

	Number	Per Cent Positive	Average Number of Microfilariae per Positive Smear
Total patients*	149		
Positive on scarification	32	21.4	22.9
Positive on thick smear	31	20.8	25.1

* All patients had both examinations at the same time. Only the first such double examination counted.

TABLE 2

Periodicity of microfilarial of W. bancrofti

Time	Average Number of Microfilaria per 20 cmm. Smear	
	Patients No. 1–11	Patient No. 12
Noon	1.1	10
1 p.m.	0.7	7
2 p.m.	0.2	2
3 p.m.	0.2	2
4 p.m.	0.9	3
5 p.m.	0.3	20
6 p.m.	2.7	26
7 p.m.	6.7	144
8 p.m.	15.0	154
9 p.m.	29.0	364
10 p.m.	29.7	274
11 p.m.	35.3	338
Midnight	31.0	309
1 a.m.	35.8	295
2 a.m.	38.6	303
3 a.m.	31.5	—
4 a.m.	32.3	254
5 a.m.	36.2	298
6 a.m.	32.7	305
7 a.m.	21.5	190
8 a.m.	11.5	72
9 a.m.	3.9	29
10 a.m.	2.5	10
11 a.m.	0.5	2
Noon	0.6	1

TABLE 3

Comparison of microfilaria counts during usual day and night examination times

Time	Average Number Microfilaria per Smear		
	Patients No. 1–11	Patient No. 12	Clinic patients
8 to 10 a.m.	5.8	37	3.0
9 to 11 p.m.	31.0	325	43.5

crofti. Six of the individuals examined had *Acanthocheilonema perstans*. No *Loa loa* was observed. The greatest prevalence and highest microfilarial counts of *W. bancrofti* were found along the coast in Marshall Territory where 34 or 9.4 per cent of 362 day and 33 or 16.4 per cent of 201 night smears were positive. Parasitemia was much less common in all localities examined in the interior of the country ranging from none at Ganta, Chuekpuzohn and Yaindawuan to 4.7 per cent at Gbanga on day examinations and from 4.5 per cent at Gbecon to 5.8 per cent at Zorzor at night (Table 4).

Almost without exception the incidence of parasitemia demonstrated in this

TABLE 4

Incidence of infection by region

Location	Day Examinations			Night Examinations		
	Total examined	Total positive	Per cent positive	Total examined	Total positive	Per cent positive
Marshall Territory near LITM	362	34	9.3	201	33	16.4
Monrovia						
City schools	497	2	0.4			
Claratown				88	6	6.8
Firestone Plantation	210	2	0.9			
Western Province						
Bomi Hills	118	2	1.7	20	3	
Zorzor				86	5	5.8
Total for Western Prov.	118	2	1.7	106	8	7.5
Central Province						
Ganta, Sanequelle distr.	39	0	0			
Gbanga	43	2	4.7			
Gbecon				67	3	4.5
Drobia				58	3	5.2
Chuekpuzohn	58	0	0			
Yaindawuan	68	0	0			
Total for Central Prov.	208	2	0.96	125	6	4.8
Total for interior locations	536	6	1.1	231	14	6.1
Total examined	1395	42	3.0	520	53	10.2

TABLE 5

Comparison of night microfilarial rates by various investigators

Location	Burch	Poindexter	Young
Monrovia (rural)	6.3	8.4	—
Marshall Territory	16.4	6.6	0.6
Robertsport	—	—	21.0
Buchanan	—	20.0	—
Greenville	—	15.0	—
Central Province	4.8	0.8	1.2
Western Province	7.5	1.0	0.9
Eastern Province	—	14.0	2.6

TABLE 6

Comparison of incidence of parasitemia and average number of microfilaria in natives from Marshall Territory and from hinterland

Region	Tribes	Day Examinations				Night Examinations			
		Total examined	Total positive	Per cent positive	Average number of microfilariae	Total examined	Total positive	Per cent Positive	Average number of microfilariae
Marshall	Coastal	254	28	11.0	7.4	141	27	19.1	53.4
Marshall	Hinterland	108	6	5.5	2.5	60	6	10.0	10.4
Marshall	All	362	34	9.4	3.5	201	33	16.4	22.5
Hinterland	All	536	6	1.1	2.1	231	14	6.1	6.8

survey was higher than that of either Poindexter or Young for the same region (Table 5). Since Monrovia was the only town where examinations were made at the same time of day or night. This can probably be explained by spotty distribution of the disease and the larger sample of blood used for smears on the present survey.

The incidence of infection among members of the hinterland tribes who had moved into the Marshall Territory was only about half that of the coast natives, but was considerably higher than that prevalent in their home regions (Table 6). Since most of these hinterland natives had only lived in Marshall Territory since the war when the U.S.A. maintained a large airbase at Robertsfield, it would appear that there has been considerable transmission of the disease in that vicinity. This may well be dependent upon the presence in Marshall of the brackish-water breeding mosquito *Anopheles melas* which has been demonstrated to have a very much higher filarial infection rate than other potential vectors (Gelfand, in press). This mosquito breeds in the brackish water swamps which extend along the entire coast of Liberia and may well account for the high parasite rates reported in other coastal areas by both Poindexter and Young.

SUMMARY

There was very little difference between the number of patients demonstrated to have microfilariae of *Wuchereria bancrofti* or in the number of microfilaria per smear as measured by scarification or by ordinary thick blood smears. Hourly thick blood smears using 20 cmm. of blood made on 12 subjects demonstrated that the most satisfactory time for making night blood examinations was between 9 and 11 pm. The concentration of microfilariae on night smears was about six times that on day smears in the periodicity examination, but about 15 times the day rate in actual practice. Forty-two or 3.0% of 1395 day smears and 53 or 10.2% of 520 night smears contained microfilariae of *Wuchereria bancrofti*. No *Loa loa* was observed but six had *Acanthocheilonema perstans*. Parasitemia due to *W. bancrofti* was much commoner along the coast than in the interior locations. This may be dependent upon the presence of the brackish-water-breeding *Anopheles melas* along the coast which has been demonstrated to have a very much higher filarial infection rate than other potential vectors.

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STUDIES ON THE VECTORS OF *WUCHERERIA BANCROFTI* IN LIBERIA[1]

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Blood smear surveys in various parts of Liberia by Poindexter (1949, 1950) revealed the widespread presence of microfilaremia caused by *Wuchereria bancrofti*, particularly in coastal areas. In a nighttime survey at Buchanan rates up to 20 per cent were recorded. More recently, Burch (1953) found rates of 7, 11, and 17 per cent in different groups of people in Marshall Territory where the work to be reported below was done. Both Poindexter (1950) and Burch (1953) have demonstrated the marked nocturnal periodicity of this parasite in Liberia.

The mosquito vectors of filariasis in Liberia have not been studied systematically. Barber, Rice, and Brown (1932) and Diller (1947) have reported finding natural infections in *Anopheles gambiae*, the latter obtaining a rate of 0.9 per cent of 649 dissected at Roberts Field. The writer became interested in this problem during the course of studies on the vectors of malaria, when filarial larvae were frequently found accidentally during dissection of the salivary glands of *Anopheles melas*. Systematic dissections of this and other mosquito species were then undertaken for the presence of filariae, and when laboratory-reared mosquitoes became available, a number of laboratory infection experiments were performed.

The present work was done from January, 1952, through April, 1953, in Marshall Territory, Liberia, in the area about the mouth of the Du River located about 30 air miles east of Monrovia along the Liberian coast. The Du River is a tidal, brackish-water stream lined for many miles by mangrove swamp. The Territory is sparsely inhabited; Marshall City, the principal town has perhaps 500 inhabitants, and the remainder of the population is scattered in many small villages consisting of two or three to several dozen huts. There are few roads, and travel is mainly by foot trails and canoe. No meteorological data are available for Marshall Territory, but these are collected at Roberts Field, located about six miles from the Du River. The total rainfall in 1952 was 188 inches, with a well marked rainy season from June through September (132 inches) and a relatively dry season from November through April (24 inches). The relative humidity remains high throughout the year, and the lowest average minimum temperature for 1952 was 71.8°F. for the month of August. For a more complete description of the area and its weather, see Gelfand (in preparation).

Elephantiasis is not common in the area. No data exist, but only a few cases have ever been seen here by the writer. Lesser clinical manifestations, perhaps

[1] This work was done as part of the United States Technical Cooperation Administration Program in Liberia and was a collaborative project of the National Microbiological Institute of the U. S. Public Health Service and the National Public Health Service of the Republic of Liberia. Laboratory facilities were made available by the Liberian Institute of the American Foundation for Tropical Medicine.

attributable to early filariasis such as inguinal lymphadenitis and pain in the scrotum, are occasionally encountered and may be associated with microfilaremia on examination. Nevertheless, the majority of blood-positive individuals give no history suggestive of clinical filariasis, and it is probable that the disease is of lesser importance when compared with other parasitic diseases present.

METHODS

The problem was approached from two directions: (1) wild-caught mosquitoes were dissected for the presence of filarial larvae resembling those of *W. bancrofti*, and (2) laboratory-reared mosquitoes of various indigenous species were fed on volunteers who demonstrated microfilariae of *W. bancrofti* in the peripheral blood. These mosquitoes were later dissected to determine the presence of filarial larvae.

Wild-caught mosquitoes were caught in a variety of ways, but all in close association with man. Two-man teams of bait-catchers worked at night with the aid of kerosene lantern and flashlight and collected, in test tubes, mosquitoes alighting on their bodies. Mosquitoes were also obtained from human-baited traps and from native huts in the morning. Day-biting mosquitoes are rare in this area. Domestic animals are not common in Marshall Territory and are rarely domiciled in human habitations; thus mosquitoes resting in huts were all presumed to have fed on man. Collections were made in a number of small villages which collectively constituted essentially a single reservoir of infection for all the species of mosquitoes reported. However, the dissections were performed over a long period of time, and the various species were not all present at any one time.

The insects were carried to the laboratory in the morning, identified if possible, and dissected. The abdomen was discarded, and head and thorax were teased apart in saline under a binocular microscope at 30×. The entire preparation was then examined at 75× under a compound microscope. In the case of the closely related species of the *Anopheles melas-Anopheles gambiae* complex, identification of eggs laid by captured females is necessary. A full discussion about relationships and differences in these two species has been presented by Muirhead-Thomson (1947) for Sierra Leone and Nigeria and by the author for Liberia (in preparation). Egg identification delayed dissection by an average of 2.9 days in each species. The age of the filarial larvae in days was estimated, and when dissection was delayed, the number of days the mosquito had spent in the laboratory was subtracted from the estimated age (except where otherwise indicated), giving the parasite age at date of capture. Females which showed only parasites which had apparently been ingested at the time of capture were recorded as "negative." The age and number of parasites were then recorded. All identifications and dissections were performed by the author.

For laboratory infection experiments, five species of mosquito were available in laboratory colonies. These were all strains originating in Marshall Territory. They were handled as follows: an "excess" cage was kept separate from the "stock" cage. Pupae in excess of those needed for breeding were placed in the "excess" cage. The emerged adults were fed on 10 per cent Karo syrup only and

permitted to mate. Twenty-four hours before an infection experiment, the syrup-water was removed. Shortly before being used, females were removed by aspirator tube and transferred to the feeding jars. These jars were improvised by removing the bottoms from one-pound jam jars and then closing both ends with cotton "mosquito netting." About 20 young females were placed in each jar through an opening made in one netting end, the cut then being sealed with adhesive tape. Mosquitoes were permitted to feed for 15 to 30 minutes by placing the jars along the thorax and thighs of the human volunteer. Feedings were either at 10 p.m. or 8 a.m. depending on the need for high or low microfilarial rates. Usually from 50 per cent to 100 per cent of the females fed, depending upon the cooperation of the human subject and the species of mosquito. The jars were then carefully examined and unengorged mosquitoes removed and destroyed. The jars were placed in a modified refrigerator-incubator where a temperature of 76 to 78°F. and a relative humidity of 70 to 80 per cent were maintained. Cotton moistened with syrup-water was placed on each jar. No undue mosquito mortality was experienced. Mosquitoes were then removed at intervals for dissection, performed as described above. Preliminary studies indicated that mature filarial larvae were first present in *A. melas* and *A. gambiae* on the thirteenth day after an infective feeding. Therefore, dissections were routinely performed on the thirteenth or fourteenth day.

RESULTS

Dissections of wild-caught mosquitoes. Table 1 summarizes the dissections performed on mosquitoes collected in Marshall Territory from January, 1952, through April, 1953. A "positive" is a female infected with filarial larvae regardless of the age or number of parasites or number of broods of worms. A "mature positive" is a mosquito with active third-stage larvae in the head or proboscis.

Table 1 also gives a very rough indication of the relative abundance of the anthropophilic mosquitoes in Marshall Territory on a year-round basis as shown by one annual cycle only. In this connection, the following comments may be made: (1) *A. gambiae* and *A. melas* alternated seasonally as the dominant mosquito. *A. gambiae*, mainly a rainwater breeder here as elsewhere in Africa, was most abundant during the rainy season; *A. melas*, which breeds selectively in residual brackish-water pools, was common only during the dry season and the early rains. (2) *A. hancocki* was very abundant during a short period of time at the end of the rains (October and November). (3) *A. funestus* was almost completely absent in this area during the period of this study, although it was abundant and an important vector of malaria in localities not over 10 miles away. In those localities, a few specimens were found infected with filarial larvae, and it is almost certainly an important vector of the disease in Liberia as it is elsewhere in Africa (Hicks, 1932; Henraard, Peel, and Wanson, 1946; Kartman, 1946). (4) *Culex fatigans* (= *C. quinquefasciatus* in the Americas) and *Aedes aegypti* were of very minor importance. This is true for the rural area of Marshall Territory only; in urban Monrovia both species were abundant and were pest mosquitoes of importance. (5) *Culex thallasius*, the most abundant anthropophilic culicine

TABLE 1

Dissections of wild-caught female mosquitoes for the presence of larvae of Wuchereria bancrofti, *Marshall Territory, Liberia, January 1952 through April 1953*

Species	Number Dissected	Total Positive		Mature Positive	
		No.	%	No.	%
Anopheles (M.) gambiae Giles	262	51	19.5	4	1.5
Anopheles (M.) melas Theo	306	83	27.1	11	3.6
Anopheles (M.) melas/gambiae complex*	592	132	22.3	11	1.9
Anopheles (M.) hancocki Edwards	50	5	10.0	0	
Anopheles (M.) hargreavesi Evans	8	0			
Culex (C.) thallasius Theo	336	2	0.6	0	
Culex (C.) fatigans Wied	23	0			
Culex (Cul.) nebulosus Theo	3	0			
Culex (C.) decens Theo	2	0			
Culex (C.) annularis Theo	1	0			
Mansonia (M.) uniformis Theo	91	4	4.4	0	
Mansonia (M.) africanus Theo	41	2	4.9	0	
Aedes (Steg.) africanus (Theo.)	30	0			
Aedes (Steg.) aegypti (L.)	29	1	3.4	0	
Aedes (Aed.) nigricephalus (Theo.) (?)	14	0			
Aedes (Aed.) punctothoracis (Theo.)	2	0			

* Dissected on the day of capture and therefore egg-identification not possible. This group is a mixture of *A. melas* and *A. gambiae*.

TABLE 2

Ages of broods of larval Wuchereria bancrofti *in infected wild-caught female mosquitoes*

Species	Age of Larval Parasites		
	First stage	Second stage	Third stage
Anopheles gambiae	11	42	14
Anopheles melas	58	59	20
Anopheles melas/gambiae	84	64	23
Anopheles hancocki	8	2	0
Culex thallasius	2*	0	0
Mansonia uniformis	4*	0	0
Mansonia africanus	2*	0	0
Aedes aegypti	1*	0	0

* No development beyond the stage of motile, exsheathed microfilariae.

mosquito in Marshall Territory, is a salt-marsh breeder found only in coastal regions. It was very abundant during the driest part of the year (December and January) and was almost completely absent during the rains. (6) *Mansonia uniformis* and *M. africanus* occurred sporadically throughout the year. (7) All other species were of negligible importance to man.

Table 2 shows the distribution of infections according to the estimated age of the parasites. Where multiple infections were present, the ages of all parasite broods are shown separately. For the purpose of this table, the age recorded is

TABLE 3

Density distribution of infections with larval Wuchereria bancrofti *in* A. gambiae *and* A. melas

Species	Total No. of Infections	Number of Larvae per Infected Female					
		1–5	6–10	11–20	21–30	31–40	41+
Anopheles gambiae	59	42 (71.2%)	5 (8.5%)	9 (15.2%)	2 (3.4%)	0	1 (1.7%)
Anopheles melas	107	38 (35.6%)	23 (21.5%)	29 (27.1%)	7 (6.5%)	6 (5.6%)	4 (3.7%)

the parasite age at the time of the dissection of the mosquito. This table shows that none of the culicine mosquitoes was infected with parasites older than two days. *A. gambiae* and *A. melas* were found with mature proboscis infections and are, therefore, natural vectors. In the small series of *A. hancocki* infections, the oldest parasites were about 10 days old.

Table 3 shows the number of parasites found per individual female *A. gambiae* and *A. melas*. For the purpose of this table, all parasites in an individual mosquito were added together regardless of possible differences in the ages of the parasites[2]. This shows the far greater percentage of low density infections in *gambiae* as compared with *melas*. The average density of parasites per infected mosquito was 6.1 in *A. gambiae* and 11.8 in *A. melas*. This difference is in the same direction as the difference in the percentages of total and mature positive in the two species.

Laboratory infections. Preliminary study was undertaken to ascertain the normal developmental cycle of *W. bancrofti* in *A. gambiae* and *A. melas* in the laboratory at a temperature of 76 to 78°F. and a relative humidity of 70 to 80 per cent. For this purpose, mosquitoes of these two species were dissected at intervals of one day until filarial maturity was noted. Development proceeded at a nearly uniform rate and in an orderly manner as described by Yamada (1927) and as illustrated by photomicrographs by Schlosser (1949). It was uncommon to find degenerating forms or individual larvae which had lagged behind their fellows by more than one or two days.

Table 4 gives a summary of dissections performed on the thirteenth and fourteenth days after feeding on the same infected human donor. This individual is a member of the Bassa tribe who has spent his entire life in Liberia. Only advanced-stage larvae (i.e., third-stage or very late second-stage) in proboscis, head, and thorax were recorded. This table shows conclusively that *A. gambiae*, *A. melas*, and *Culex thallasius* are readily infected and able to carry the infection to maturity in the time allowed. It also shows that *Culex fatigans* and *Aedes aegypti* are susceptible to infection to a much lesser degree, and that, in both, the develop-

[2] This has caused a discrepancy in the total number of infections as shown in Tables 1 and 3. In Table 1 mosquitoes which demonstrated developing parasites in the thorax only as a result in the delay in dissection necessitated by oviposition were recorded as "negative." In Table 3 all parasitized mosquitoes were included, and the totals are therefore greater.

TABLE 4

Dissections of laboratory-infected mosquitoes, performed on the thirteenth or fourteenth day after the infective feed, showing the number of advanced-stage filarial larvae

Species	Microfilarial Counts in Human Donor per 20 cu.mm. Blood						
	13	20	63	89	98	113	116
Anopheles gambiae							
No. dissected	29	36	30	35	24	51	
No. positive	5	9	10	21	12	28	
% positive	17.2	25.0	33.3	60.0	50.0	55.0	
ADP*	1.4	1.6	2.7	3.3	2.9	4.6	
Anopheles melas							
No. dissected		41			19	33	25
No. positive		10			11	24	14
% positive		24.4			57.9	72.8	56.0
ADP*		1.4			5.3	4.0	7.0
Culex thallasius							
No. dissected	54						
No. positive	23						
% positive	42.6						
ADP*	2.7						
Culex fatigans							
No. dissected		35	22	33			9
No. positive		1†	0	4‡			1†
Aedes aegypti							
No. dissected	25	57			28	13	38
No. positive	1†	0			0	0	0

* ADP = average density of parasites per infected female.

† In each case, one mosquito contained one second-stage parasite in the thorax.

‡ One female with one second-stage parasite, two with one third-stage larva each, and one with two third-stage larvae in the head.

mental cycle of the filarial larvae tends to be longer than fourteen days. Dissections of infected individuals of these latter two species on the fifth and seventh days after infection showed the presence of many immature larvae (one, two, and three days of estimated age) but no development beyond this point except in the examples shown in the table. In *Culex thallasius* also, in addition to the filariae of the expected age, many immature and degenerating worms were seen. In these three culicine species, therefore, *W. bancrofti* larvae successfully reach the thoracic muscles, but many of the parasites are subsequently unable to complete development. This is in marked contrast to the two anophelines studied, where immature and degenerating worms were rarely noted.

DISCUSSION

Dissections of wild-caught mosquitoes have demonstrated the presence of mature natural infections with *Wuchereria bancrofti* in *A. gambiae*, *A. melas*, *A. funestus*, and *A. hancocki*. All of these species are at least locally abundant in Liberia, and hence may be of actual importance in the transmission of Bancroftian filariasis in that country. In Marshall Territory during the one-year period

of this study, *A. funestus* was very rare, and *A. hancocki* was present in numbers only during a short period of time. *A. gambiae* and *A. melas* alternated as the predominant species; *gambiae* is therefore probably the main vector of filariasis in this area during the rainy season, and *melas* is probably the main vector during the dry season and early in the rainy season.

Because the dissections of *A. gambiae* and *A. melas* were not made at the same time and were not analyzed for their relation to total population, it is possible that the observed differences in infectivity rates might have been due to selecting the individual mosquitoes during a phase of population rise or fall, and therefore with a preponderance of younger or older specimens in the sample. However, dissections of the salivary glands of the same mosquito specimens for malaria sporozoites (Gelfand, in preparation) produced a reverse relationship, i.e., an infectivity rate of 5.7 per cent for *gambiae* and 1.4 per cent for *melas*, suggesting a real difference in the practical ability of the two mosquito species to transmit the two diseases.

The results of dissections of wild-caught specimens of *A. gambiae* and *A. melas* can be compared with the results of laboratory infections in the two species in the following table, which is based on Tables 1, 3, and 4:*

	Wild-caught Mosquitoes		Laboratory Infections†	
	A. gambiae	*A. melas*	*A. gambiae*	*A. melas*
Per cent infected	(51/262) 19.5 ± 2.4‡	(83/306) 27.1 ± 2.5	(61/110) 55.5 ± 4.7	(49/77) 63.6 ± 3.7
Average number of parasites per infected female	(360/59) 6.1 ± 1.0	(1259/107) 11.8 ± 1.1	(233/61) 3.8 ± 0.5	(253/49) 5.2 ± 0.7

* The assistance of Mr. Nathan Mantel, of the National Cancer Institute, National Institutes of Health, for the statistical interpretation, is gratefully acknowledged.

† Based on feedings on donor blood containing an average of five microfilariae per cubic millimeter (89–116 per 20 cu. mm.).

‡ Standard error is given for all rates.

In the series of dissections of wild-caught females, the differences between the rates for *A. gambiae* and *A. melas*, both for the percentage infected and the average parasite density, are statistically significant. For laboratory-infected females, the differences between the species are qualitatively in the same direction but taken alone are not significant. Both series are small and must be extended before the results can be considered conclusive. They appear to show, however, that *A. melas* is more heavily infected with the intermediate stages of *W. bancrofti* in nature than is *A. gambiae*, although the converse is true for infections with malaria. The data from laboratory infections do not adequately explain this difference.

In all the dissections on which this paper is based, the mosquito abdomen was discarded. During the latter part of the experimental work, it was realized that this was causing a substantial error in both the percentage infected and the num-

ber of worms per infected female (see Pratt and Newton, 1946). To evaluate the degree of error thus introduced, in 25 laboratory-infected *A. gambiae* the abdomen was dissected separately and a record kept of the number of parasites in that region and in the entire body. In this series, by dissecting the abdomen the total number infected was increased by one individual (from 56% to 60%) and the average number of parasites per infected female from 7.0 to 10.3. Although very important, this error was present for both *gambiae* and *melas*.

The failure to find advanced-stage filarial infections in wild-caught *Culex thallasius* was somewhat surprising in view of its ready infectability in the laboratory and the readiness with which it was found to feed upon man both in the field and in the laboratory. This may possibly indicate that man is not the most frequent host of this mosquito. The series was limited, however, and more work will have to be done to evaluate this properly.

SUMMARY

From January, 1952, through April, 1953, a series of dissections of wild-caught anthropophilic mosquitoes for larval worms resembling *Wuchereria bancrofti* was performed in Marshall Territory, Liberia. Advanced-stage infections were found in *Anopheles gambiae*, *A. melas*, and *A. hancocki*. In addition, natural infections have been found in *A. funestus* in a nearby locality in Liberia. No advanced-stage infections were found naturally in any culicine mosquito.

Five species of laboratory-reared indigenous mosquitoes were fed on a human donor with microfilariae of *W. bancrofti*. *A. gambiae*, *A. melas*, and *Culex thallasius* were readily infected and carried the infection to maturity within thirteen to fourteen days. *Culex fatigans* and *Aedes aegypti* became infected in a much lower percentage, and development of the worms tended to be slower. In all three culicine species, many young nondeveloping larvae were noted in the thoracic muscles.

In the small series reported here, *Anopheles melas* was more heavily infected with the intermediate stages of *W. bancrofti* in nature than *Anopheles gambiae*.

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THE EFFECT OF CORTISONE ON THE IMMUNE RESPONSE OF THE WHITE RAT TO *NIPPOSTRONGYLUS MURIS*

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Cortisone and adrenocorticotropic hormone (ACTH) have been shown to have some effect in alleviating severe clinical symptoms associated with *Trichinella spiralis* infection in man and the guinea pig (Davis and Most, 1951; Luongo, Reid and Weiss, 1951; Rothenberg, 1951; Rosen, 1952; Faiguenbaum, 1953), as well as in reducing the severity of the sensitization reaction of the guinea pig to *Ascaris* extract (Studer and Fust, 1951). However, Galliard and Berdonneau (1953) have reported that an old dog which previously was refractory to *Strongyloides stercoralis* infection, became susceptible after treatment with cortisone. In addition, Stoner and Godwin (1953) have demonstrated that ACTH and cortisone did not prevent the deaths of mice from severe trichinosis when the animals were given a large infective larval dose, and McFadzean (1953) found that ACTH had no effect on elephantiasis of a limb resulting from *Wuchereria bancrofti* infection.

In view of some of the encouraging results already obtained in trichinosis in man, the use of ACTH and cortisone may become more frequent in attempts to control the marked sensitization and inflammatory reactions which occur in other helminth infections. It is possible, however, that in certain of these infections, if the inflammatory response were suppressed by the use of these hormones, larvae which ordinarily might be trapped in the tissues would now have the opportunity to continue their migration and development. *Nippostrongylus muris* infection in the white rat offered a convenient tool to test this hypothesis. One of the important expressions of the immune response in the white rat to infection with this nematode is the development of an inflammatory reaction which is directed against the larval and adult stages of the worm. The sequence of events in the development of this response to migrating larvae in the skin and lungs, and to adult worms in the intestine has been described in considerable detail by Taliaferro and Sarles (1939). In addition, the appearance of circulating antibody which is protective in nature has also been demonstrated (Sarles, 1938; 1939; Taliaferro and Sarles, 1942). Apparently operating together the cellular response and action of antibody in the immunized animal result in the entrapment of larvae in the tissues through which they migrate, as well as in the stunting of adult worms in the gut. In the following experiments cortisone was administered in an attempt to determine whether it would alter the pattern of this immunological picture. A brief report summarizing the findings has been published previously (Weinstein, 1953).

MATERIALS AND METHODS

Experiment 1. Sprague-Dawley female rats 8 weeks old were divided into 5 groups and treated as follows (Table 1).

TABLE 1

Schedule of immunizations to Nippostrongylus muris *and cortisone treatments of rats in experiments 1 and 2*

Group	Larval Exposures			Cortisone		
	Day	No. larvae		Days treated	Dosage (mg. per day)	
		Exper. 1	Exper. 2		Exper. 1	Exper. 2
A	1	300	300	None		
	5	1,000	1,000			
B	12	2,000	2,000	−4 to necropsy	2	2
	18	1,000	1,000			
C	24	1,000	2,500	26 to 30	5	2
	31	1,000	1,000	31 to necropsy	2	2
D	31	1,000	1,000	None		
E	None			None		

a. *Larval exposures.* Filariform larvae of *N. muris* were isolated from charcoal cultures and washed by several sedimentations through sterile saline. Immunization was accomplished by injecting known numbers of larvae subcutaneously into the dorsum. To obtain information on the skin response of immunized and control rats to larval penetration, the final challenge exposure was made on these animals in the following manner. The rats were anesthetized with nembutal, the abdominal hairs clipped with an electric shaver and the larval suspension applied to the skin in a small amount of water on a confined area marked with India ink. The immunization schedule consisting of 5 injections of larvae and a final percutaneous exposure on abdominal skin is given in Table 1. Rats in groups A, B, and C received equally all the immunizing and abdominal larval exposures; group D received the abdominal exposure only and was not given any of the immunizing doses, and group E was not infected with any larvae at all, but a sham abdominal exposure was made as a control on the possible irritative effect that the electric shaver might have on the skin.

b. *Cortisone treatment.* Group A. Ten animals were given daily saline control injections only and did not receive cortisone.

Group B. Six animals were each given daily 2 mg. cortisone acetate (Cortone, Merck) subcutaneously in the dorsum, beginning injections 4 days prior to the first immunizing exposure to larvae and continuing until the day of necropsy.

Group C. Ten animals were given saline injections daily, but for the 5 days prior to the abdominal exposure to larvae they received 5 mg. cortisone daily. After this larval exposure, 2 mg. cortisone daily was given until necropsy.

Groups D (6 rats) and E (4 rats) received daily saline control injections but did not receive cortisone at any time.

c. *Skin biopsies.* On day 33, approximately 48 hours after the percutaneous penetration of larvae through the abdominal skin, all animals in groups A, B, C, D, and E were anesthetized, and the entire area which had been exposed

was removed and the wound closed with metal clips. From each biopsy 150 serial sections were prepared and stained. All sections were carefully examined for the presence of larvae and for indications of an inflammatory reaction.

d. *Necropsy.* Thirteen days after the abdominal exposure to larvae all animals were anesthetized, bled from the heart, and the lungs removed and fixed in Bouin's fluid. Various tissues were taken for examination as fresh press preparations for the presence of larvae. The entire intestine was then placed in saline and refrigerated until examined. To recover all the adult worms, the intestine was slit open in a dry Petri dish and under a magnification of 6.3 diameters the worms present were counted as they were teased into the dish. *N. muris* adults are extremely difficult to measure with any degree of precision because the living worms become tightly spiraled when handled (Lindquist, 1950). It was found, however, that accurate measurements on unfixed worms could be made in the following manner. Worms which had been teased from the opened gut in making the total count were collected in a pool of mucus. Under a magnification of 20 diameters using finely pointed needles, a worm was removed with a small amount of mucus onto a clear area in the dish and carefully uncoiled and straightened by teasing it along the sticky surface of the dish. Undistorted specimens were obtained in this manner which were measured immediately with a calibrated ocular micrometer. In most instances, 15 male and female worms each were taken at random for measurement from each rat.

e. *Serologic examination for antibody.* Tests for the formation of precipitate at the excretory pore of filariform larvae incubated *in vitro* in serum were carried out according to the method of Sarles (1938). To avoid the possibility of bacterial interference, duplicate preparations were also made in serum to which penicillin and streptomycin were added following the recommendations of Smith (1953) and Thorson (1953).

f. *Maintenance of animals.* All rats were kept on wire mesh in individual suspended cages, fed Purina Laboratory Chow supplemented with greens, and received water *ad libitum.*

g. *Statistical tests.* The significance of observed differences between groups was determined using Student's "t" test. A probability less than 0.05 was considered significant. Because the means and standard deviations were proportional to each other, it was considered worth while to conduct the tests of significance based on the square root of the counts (Snedecor, 1946). The author wishes to thank Mr. Marvin Schneiderman of the Biometrics Section, National Cancer Institute, for advice on the statistical treatment.

Experiment 2. This experiment essentially repeated the first, but one important modification was introduced. Although no continuous weight records had been kept on the animals in the first experiment, it was noted that the ones on cortisone treatment did not show weight gains comparable to the other groups. Since certain dietary deficiencies have been demonstrated to increase susceptibility to *N. muris* infection (Spindler, 1933; Donaldson and Otto, 1946), there was the possibility that retarded weight gain might have entered as a factor. Consequently, in the second experiment, weights of all animals were

followed throughout the course of the study, and those not on cortisone had their diet restricted in quantity so that their weight gains coincided fairly closely to those of the rats treated with the hormone.

Five groups of rats were set up to correspond to groups A, B, C, D, and E of experiment 1 with regard to cortisone treatment and larval exposures. There were 11 animals each in the first 4 groups, and 6 animals in the last group. It was not possible at this time to repeat the great number of serial sections of skin prepared in experiment 1. Results were evaluated primarily on the mean number and size of adult worms recovered from each group of rats.

Immunization to larvae was conducted as before with the exception that the last exposure was also injected, since no skin biopsies were to be performed. The numbers of larvae and the time interval between exposures were the same as before except that for the fifth exposure 2,500 larvae instead of 1,000 were injected, and that necropsy took place 11 days after the last larval exposure.

Cortisone was given in the same amount and followed the same schedule as in experiment 1, with the exception that for group C in which cortisone treatment was first started 5 days prior to the final larval exposure, 2 mg. daily instead of 5 were given during this period, but, as in the first experiment, the animals then received 2 mg. daily until necropsy.

RESULTS

The animals in groups A which received all the immunizing larval exposures but were not treated with cortisone developed an extremely strong immune reaction in both experiments, as measured by the entrapment of larvae in the inflammatory responses in skin and lungs, the marked stunting of adult worms in the gut, and the presence in the serums of antibody causing formation *in vitro* of a precipitate at the excretory pore of exposed larvae. The modifications in the immune response of animals treated with cortisone were measured against these findings.

Histopathological response. Skin. From rats in experiment 1, in groups A, B, C, D, and E, totals of 1,500, 900, 1,350, 900, and 600 serial sections respectively

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PLATE I

All sections photographed at a magnification of 42 diameters

FIG. 1. Normal rat skin (group E).

FIG. 2. Skin from rat given one larval exposure only (group D). Slight inflammatory reaction present; no larvae found in these skins.

FIG. 3. Skin from immunized rat not treated with cortisone (group A). An intense inflammatory response with tremendous thickening of skin due to edema and cellular infiltration has occurred. Note that this section was photographed at the same magnification as all the others. Larva (arrow) is trapped in large nodule which has formed around it.

FIG. 4. Skin from immunized rat started on cortisone prior to first exposure to larvae, and continued on cortisone all during the immunization procedure (group B). Note lack of inflammatory response, and absence of larvae. Some atrophy apparent.

FIG. 5. Skin from rat which was immunized and then treated with cortisone beginning a few days prior to challenge exposure (group C). As in figure 4, note lack of inflammatory response, absence of larvae, and close similarity to normal skin (fig. 1.).

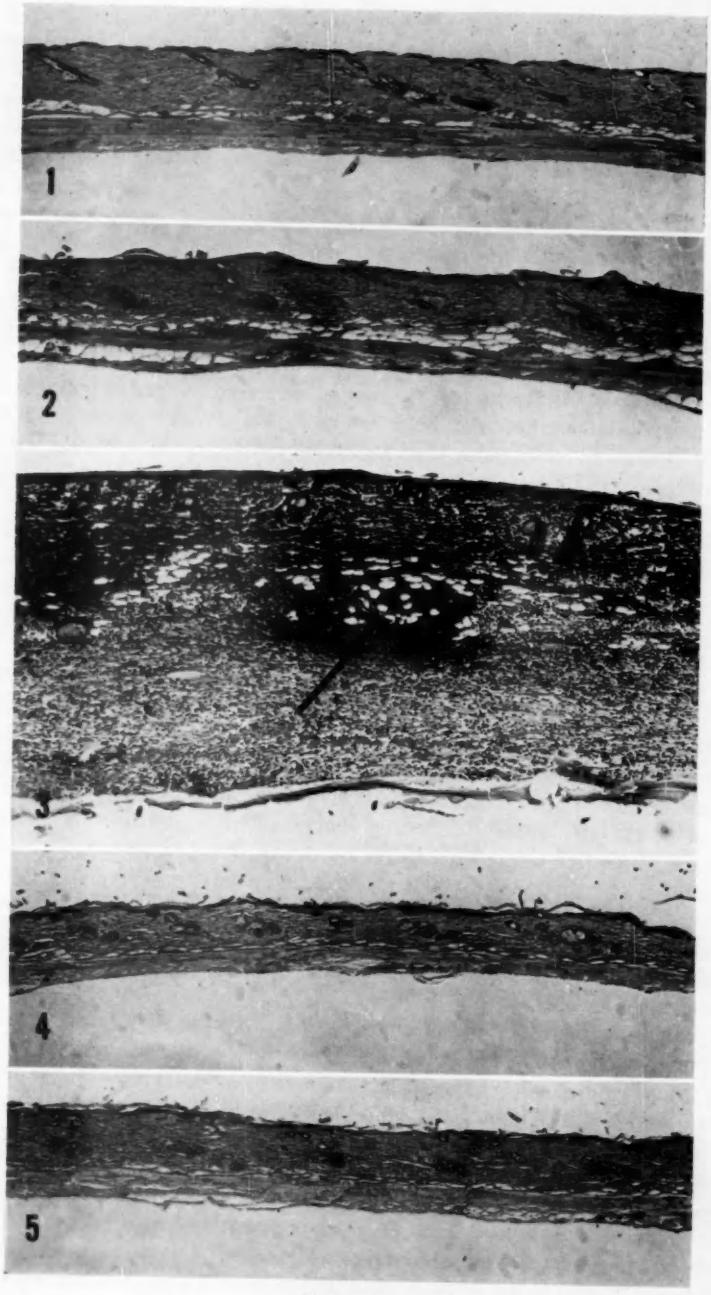

PLATE 1

were examined. Skins from normal unexposed control animals (group E) showed virtually no cellular infiltration, and edema was not present. The tissues were normal in all respects (Fig. 1). In the skins of rats which had received only a single larval infection (group D), no larvae were found and only a minimal inflammatory response was noted which consisted of small scattered accumulations of leucocytes (Fig. 2). The skin sections from animals in group A, however, showed in most of them the typical reaction of the highly immunized rat as described by Taliaferro and Sarles (1939). An intense inflammatory response with nodule formation and considerable edema was present in 8 of the 10 biopsies obtained from this group (Fig. 3). In 7 of these 8, many larvae were found trapped within the nodules; a total of 54 worms was counted. The 2 other biopsies from this group showed only a moderate cellular infiltration; no large nodules or larvae were present.

An impressively different picture was encountered in studying the sectioned skins from animals that had been on cortisone throughout the immunizing process (group B), and those that had been treated with cortisone starting 5 days prior to the challenge exposure of larvae to the abdominal skin (group C). A striking suppression of the inflammatory reaction occurred in both these groups, each presenting essentially the same picture. All skins except one showed little cellular infiltration other than the occasional occurrence of small accumulations of leucocytes, and edema was not apparent. Of particular interest was the fact that not a single larva was encountered in these 15 biopsies. As illustrated by photomicrographs of representative sections (Fig. 4 and 5), skins from these 2 groups were remarkably similar in appearance to those obtained from normal animals. In one, however, an animal from group B, an inflammatory response was evident which was diffuse in nature with one nodule encountered; the nodule did not contain a larva. There were indications of slight atrophic changes occurring in the skins of some of the cortisone-treated animals.

Other tissues. An interesting finding concerned the appearance of milky-white spots, each about 1 mm. in diameter, on the peritoneal membranes supporting the duodenum and pancreas, and a few such spots in the livers of animals in groups A, B, and C but not in D or E. When such a spot was excised and mounted between slide and coverslip as a press preparation, it was found to contain a larva trapped within a nodule (Fig. 6); many of these organisms were still alive and moving actively.

Larvae were found in the sections of the lungs of all immunized animals, whether treated with cortisone or not. These larvae were mostly found caught in an intense inflammatory infiltration, though a few were seen apparently lying free in an alveolus or among the septal cells. No marked differences were noted with regard to the degree of inflammation surrounding larvae in lungs of immunized animals treated with cortisone as compared to those not so treated. Larvae were not encountered in the lungs of animals which received only a single larval infection, and these lungs showed little sign of inflammation.

Adult worm counts. As indicated under "Methods," in experiment 2 the animals not given cortisone had their diet restricted in quantity so that the weight gains

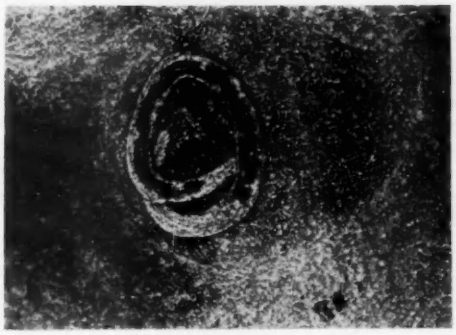

FIG. 6. Fresh press preparation of liver nodule showing larva trapped in an inflammatory reaction (× 125).

of all groups would be approximately the same. The initial mean weights of groups A, B, C, D, and E were 139, 138, 138, 137, and 138 gm. respectively, and the final mean weights at necropsy were 162, 165, 157, 167, and 161 gm respectively. This weight control appeared to have little effect on the results obtained, since the over-all findings of experiments 1 and 2 are fundamentally the same (Table 2). Groups A and B are of chief interest for comparison, the former representing animals immunized but not treated with cortisone, while the latter represent rats treated with cortisone continuously during the immunizing procedure. In both experiments, group B showed a significantly greater number of worms developing to the adult stage. Group C had undoubtedly reached a high level of immunity before cortisone treatment was begun; in neither experiment was a significantly greater number of worms recovered from this group as compared to group A. No worms were encountered in the unexposed groups E, and the infection rate for the single exposure in the control groups D was very similar for the two experiments.

Lengths of adult worms. Considerable stunting of adult worms occurred from the immunizing procedure in both experiments 1 and 2, although treatment with cortisone tended to reverse this effect (Table 2). In groups A, the mean lengths of the male and female worms were significantly smaller than those of the worms from groups D which received only a single exposure to larvae. The ones from the latter groups fell within the normal size range for the species (Yokogawa, 1922; Lindquist, 1950). However, for each of the 2 groups treated with cortisone (B and C), the male and female worms were longer than the ones from group A though smaller than the normal sized worms from group D. As regards the two different cortisone regimens (groups B and C), from the rats on continuous cortisone (group B), the male worms in both experiments were significantly greater in length than those from rats in group C; however, for the females this was only true in the first experiment.

Excretory pore precipitate formation in serum in vitro. Since this test does not readily lend itself to quantitative work, the purpose of the experiment was only

TABLE 2

Effect of cortisone on the numbers and lengths of adult Nippostrongylus muris *recovered from immunized white rats and controls*

	Experiment 1				Experiment 2			
Group	A	B	C	D	A	B	C	D
No. rats	10	6	10*	6	11	11*	11	11
Mean no. worms	485 ± 131†	1,156 ± 236	492 ± 78	251 ± 70	119 ± 50	582 ± 101	178 ± 42	236 ± 37
Range	137–1,636	506–2,039	141–890	19–538	8–595	204–1,139	10–423	106–540
Males, mean length, mm.	2.53 ± 0.04	3.20 ± 0.04	3.04 ± 0.03	3.43 ± 0.04	2.70 ± 0.04	3.02 ± 0.05	2.84 ± 0.04	3.70 ± 0.02
Range	1.22–3.60	2.16–4.03	2.30–3.74	2.30–4.18	1.58–4.03	1.00–4.25	2.09–4.03	2.95–4.39
No. measured	124	86	134	91	144	150	148	165
Females, mean length, mm.	2.85 ± 0.05	3.71 ± 0.08	3.57 ± 0.04	4.44 ± 0.06	2.74 ± 0.04	2.96 ± 0.06	3.09 ± 0.04	4.80 ± 0.03
Range	1.87–4.82	1.37–5.04	2.52–5.04	3.38–5.62	1.66–5.11	1.66–4.75	2.16–4.75	4.03–6.05
No. measured	140	90	134	70	144	150	144	165

A. Received all immunizing larval exposures and challenge exposure; no cortisone given.

B. Received all immunizing larval exposures and challenge exposure; cortisone given 2 mg. daily beginning 4 days prior to first immunizing exposure and continued until necropsy.

C. Received all immunizing larval exposures and challenge exposure. In experiment 1, 5 mg. cortisone daily was started 5 days prior to challenge exposure, following which the animals were given 2 mg. daily until necropsy. In experiment 2, 2 mg. cortisone daily was started 5 days prior to challenge exposure; this dosage was continued until necropsy.

D. Received single larval exposure only (challenge exposure); no cortisone given. In experiment 1, larval exposure performed by applying larvae to skin and allowing them to penetrate; in experiment 2, the organisms were injected subcutaneously.

* One died; not included in results.

† Figure following ± is standard error.

Significance tests: The difference between groups is considered significant if $P < 0.05$.

Worm counts. Experiment 1. Groups A and B, $0.01 < P < 0.02$; B and C, $0.01 < P < 0.02$; A and C, $P > 0.9$. Experiment 2. Groups A and B, $P < 0.01$; B and C, $P < 0.01$; A and C, $0.3 < P < 0.2$.

Worm lengths. Experiment 1. Male worms. Groups A and B, A and C, B and C, B and D, A and D; $P < 0.01$ respectively. Female worms. A and B, A and C, B and D, A and D; $P < 0.01$ respectively. B and C $0.01 < P < 0.02$. Experiment 2. Male worms. Groups A and B, A and C, B and C, B and D, A and D; $P < 0.01$ respectively. Female worms. A and B, A and C, B and D, A and D; $P < 0.01$ respectively. B and C, $0.1 < P < 0.05$.

to determine whether the antibody would be present at all in serum from immunized animals treated with cortisone. In experiments 1 and 2, in the serums from the immunized group (A) as well as from the immunized and cortisone-treated groups (B and C), excretory pore precipitate was formed in all instances and was similar to what has been described by Sarles for serum from highly immunized animals. Large irregular plumes of refractile material formed and remained attached at the excretory pore, many eventually breaking free. Although the amount of precipitate, both adherent to the larvae and free in the serum, varied somewhat from serum to serum, most showed strong reactions and no obvious differences were noted when the three groups were compared with one another. Serums from most of the animals which received only a single exposure to larvae (groups D) showed precipitate formation but definitely much less in amount than in the three immunized groups. It usually occurred as small blebs at the excretory pore instead of in long streamers, and relatively little precipitate appeared free on the slide. Excretory pore precipitate did not form in any of the serums from animals not exposed to larvae (groups E), and the serums themselves remained clear.

The addition of penicillin and streptomycin to duplicate preparations did not change the readings of these tests in any way. However, the occasional bacterial growth which developed when the antibiotics were not used was now completely suppressed, and it was felt that greater reliability could be placed upon the results.

DISCUSSION

The action of large doses of cortisone or ACTH in increasing the susceptibility of a host to a parasite, as well as the role of these hormones in activating latent infections, has been described with regard to a wide variety of biological agents, including bacteria, protozoa, and viruses (Thomas, 1953; Shwartzman, 1953; for reviews).

The results of the present study indicate that under certain circumstances nematode infections may behave in a similar manner. The underlying mechanisms by which cortisone affects the host-parasite relationship are not well understood, but some of the effects concern the suppression of the inflammatory response, the reduction of antibody titer under certain conditions, and the retardation of host growth with accompanying metabolic changes. Cortisone suppressed the inflammatory response in the skin to *Nippostrongylus* larvae not only in animals given the hormone throughout the immunizing process but also in those treated for the first time only a few days prior to the larval challenge and after a high degree of immunity had already been reached. No larvae were found in the skin biopsies of these animals. Whether the slight atrophic changes seen in the skin of animals under continuous cortisone treatment in some way contributed to the lack of reaction of this tissue to invading larvae cannot as yet be evaluated. Such atrophic skin changes have been described for normal rats exposed to large doses of cortisone (Winter, Silber and Stoerk, 1950; Selye, 1953).

The pattern of observations in the present study parallels those described for the action of cortisone on inflammation induced by bacteria or their products. Robinson and Smith (1953) have shown a delay in mobilization of neutrophilic leucocytes in the skin of rabbits infected with pneumococci and treated with cortisone, and Rebuck and Mellinger (1953) have demonstrated in man that the topical application of cortisone to a skin lesion stimulated with diphtheria toxoid as an inflammatory agent resulted in retardation and incompleteness of the cellular response, particularly with regard to lymphocytic participation.

In contrast to the skin findings in the present experiments, larvae were trapped in a considerable cellular response in the lungs, liver, and peritoneal membranes in all the immunized groups whether treated with cortisone or not. (This is apparently the first report of migrating *Nippostrongylus* larvae being encountered in organs other than the skin and lungs in the white rat; we have subsequently found this to occur in several other unrelated experiments.) In all instances these probably represent larvae from several different exposures. Apparently then, there was not an absolute inhibition by cortisone of the inflammatory reaction to migrating larvae, but a considerable suppression and slowing up of the process. This allowed most, if not all the larvae to penetrate the skin barrier successfully, but as they continued their migrations, some eventually did become trapped in a local tissue reaction. The mechanism and sequence of events in this complex immune relationship are still rather obscure (Lindquist, 1950; Thorson, 1953). It is possible, however, that as the larvae were exposed to circulating antibody during migration, many became inhibited in their progress so that the cellular response, even though considerably repressed, eventually had sufficient time to develop around them. The data indicate that this occurred to a lesser degree in the cortisone-treated than in the non-treated rats, since the significantly greater number of adult worms which were found in the intestine of animals that received the hormone throughout the immunization period in both experiments is undoubtedly a reflection of this partial breakdown of the immune response. The larger size of the worms in these rats also strengthens this hypothesis. This greater number of worms can probably be explained as a cumulative process, each exposure in the cortisone-treated animals resulting in a somewhat higher number of adults developing than in the non-treated group so that the final total was greater.

No significant difference in the number of adult worms, however, was demonstrated between those rats given cortisone for the first time just prior to the challenge dose of larvae after immunization had already reached a high level and the immunes not treated with the hormone (groups A and C). The evidence indicates, though, that a partial suppression of the immune response did occur, based on the facts that the inflammatory response in the skins was virtually lacking with no larvae being trapped there, and the size of the adult worms recovered from these animals was larger as compared to the non-treated group. It was to be expected that unless the immune response were reduced almost to zero by cortisone, it would be difficult to demonstrate whether real differences existed between total numbers of adult worms recovered from these two groups.

As already postulated from the data obtained from the animals on continuous hormone treatment while being immunized (group B), it is probable that the number of worms which they developed from any one given larval exposure was in excess of that recovered from the non-treated immune group (group A). It is not possible to state, however, from the design of the present experiments whether these differences were significant for each of the exposures.

A few interesting reports have appeared concerning the effect of cortisone or ACTH on the tissue response to other nematodes, but no consistent pattern has emerged as yet. Studer and Fust (1951) sensitized guinea pigs to *Ascaris* extract and found that the clinical symptoms of shock in the animals exposed by inhalation were less marked in those which had had a previous course of cortisone injections. Compared to the control animals, the ones treated with cortisone showed at the sites of the injection a lessened tissue eosinophilia, though the leucocytic and later round cell responses were uninfluenced. On the other hand, Luongo, Reid and Weiss (1951), and Stoner and Godwin (1953) have reported no detectable difference between the tissue reactions of laboratory animals infected with a single dose of *T. spiralis* larvae and treated with ACTH, and similarly infected but untreated animals.[1] The former investigators, however, stated that there was little inflammatory response in either group, while the latter gave no details. In a case of human trichinosis which responded clinically in a satisfactory manner to treatment with ACTH started the third week of illness, there was considerable inflammatory reaction to larvae in muscle biopsies taken on the ninth and twelfth days of treatment (Davis and Most, 1951).

Although in the present study no quantitative differences were sought in antibody production between the cortisone-treated and nontreated groups of immunized rats, from the large amount of excretory pore precipitate formed in all of these groups as compared to the relatively small amount of precipitate formed in serums from the group receiving only a single larval infection, it is believed that cortisone had either no or only little effect on the titer of this particular antibody. Of interest is the report of Davis and Most (1951) that the complement-fixing antibody titer to *T. spiralis* continued to rise during the period in which their patient was treated with ACTH.

The interesting effect of adrenalectomy on the natural and acquired resistance of mice to *Trichinella spiralis* has recently been reported by Baughn (1952). He found that adrenalectomized mice previously unexposed to the nematode, and maintained on desoxycorticosterone acetate, demonstrated a greater resistance to infection than did the controls. Also, that immune mice which were adrenalectomized showed a resistance above that manifested by immunized mice alone. In light of the present study and that of Stoner and Godwin (1953), it would be of interest to determine whether cortisone alone administered to such adrenalectomized animals would tend to reverse these results, and whether the magnitude of reversal would be a function of dosage.

[1] After this paper went to press, Pollay, Wein and Hartmenn (*Proc. Soc. Exper. Biol. Med.* **86**: 577–580, 1954) reported that there was a considerable reduction in the inflammatory response of rats infected with *Trichinella spiralis* and treated with ACTH.

The data of the present report indicate that with high doses of cortisone, a partial breakdown of the immune response to a nematode infection can occur under laboratory test conditions. This phenomenon under certain circumstances may have a bearing on the clinical use of cortisone in helminth infections in man. Galliard and Berdonneau (1953) have reported that under cortisone treatment, an old dog previously immune to *Strongyloides* infection became susceptible, and Stoner and Godwin (1953) have obtained a higher mortality rate in mice given one exposure to *T. spiralis* while treated with cortisone as compared to infected mice not given the hormone.

The effectiveness of cortisone in the treatment of infections due to bacterial, viral, and protozoan organisms is greatly diminished because of the uncontrolled and overwhelming multiplication that usually occurs within the host. Despite the contraindications of the present report, it should be pointed out that the pathologic effects of helminthic infections may be peculiarly more suited for approach with the corticosteroids than are the infections due to unicellular organisms, since in most instances there will be no danger of the actual *multiplication* within the host of the helminths following administration of the hormone. It is probably for this reason that of the few reports of the successful use of cortisone and ACTH alone to control the effects of an infecting agent, most of these have been of cases of helminthiasis (trichinosis); few have involved unicellular organisms (Overman, Bass, Davis, and Golden, 1949; Robinson, Mason and Smith, 1953).

Further experimental work on laboratory animals should help delineate the conditions under which the use of these hormones in helminthic infections may prove most beneficial, and also wherein their dangers may lie.

SUMMARY

Cortisone administered to white rats while they were being immunized to *Nippostrongylus muris* resulted in suppression of the inflammatory response in the skins of these animals when subsequently challenged with larvae, and apparently all of the larvae successfully penetrated through this first barrier. Many, however, were trapped in a cellular reaction in the lungs, liver, and peritoneal membranes during their migrations, but a greater number matured to adults and were larger in size than in the nontreated immune controls.

Rats immunized and then treated with cortisone for the first time a few days prior to the skin challenge with larvae also demonstrated some reduction in the over-all immune reaction; this was manifested by a suppression of the cellular response in the skin but not in the internal organs, and by an increased mean length of the adult worms as compared to the nontreated immune controls. However, there was no significant difference in number of adult worms developing in this group as compared to the controls.

Filariform larvae incubated in serums obtained from all immunized groups, whether they had been treated with cortisone or not, showed excretory pore precipitate formation which was considerable in quantity.

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SUSCEPTIBILITY STUDIES IN SCHISTOSOMIASIS. II. SUSCEPTIBILITY OF WILD MAMMALS TO INFECTION BY *SCHISTOSOMA MANSONI* IN EGYPT, WITH EMPHASIS ON RODENTS[1]

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Most of the commonly used laboratory animals have been subjected to infection by *Schistosoma mansoni* and *S. japonicum*. The earlier investigations in experimental schistosomiasis were concerned primarily with the life cycle and development of *S. japonicum* (Faust and Meleney, 1924). Later studies of a similar nature were followed employing *S. mansoni* in Puerto Rico (Faust *et al.*, 1934). Also, Leiper (1915) in his attempt to elucidate the schistosome life cycle in Egypt used available laboratory animals to obtain adult worms to differentiate *S. mansoni* from *S. haematobium*. More recently the susceptibility of various animals to schistosome infection has become of concern because of the accelerated interest in schistosomiasis during and following World War II.

In general, suceptibility studies have been made in an effort to evaluate the usefulness of various hosts for investigation of different phases in the biology of the schistosomes under laboratory conditions. Special attention has been directed toward the use of small animals which take infections readily and thus provide for working quantities of schistosome materials, and as a means for the screening of drugs in chemotherapy studies. Another consideration has been hosts which manifest pathological conditions similar to those found in schistosome infections in man. Further, a knowledge or evaluation of relative susceptibilities has indicated the hosts that may be employed in considering immunity and host-resistance mechanisms.

Attempts to infect wild mammals with *S. mansoni* and *S. haematobium* have been few and as a rule have been incidental to other studies in the field or the laboratory. The present investigation was undertaken in Egypt to learn which of the wild mammals are capable of taking schistosome infection. In the earlier part of this work emphasis was given to infection of those species of animals which normally are found in the rural districts of Egypt where *S. mansoni* and *S. haematobium* are endemic. Knowing the relative susceptibilities of these hosts

[1] The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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The authors are indebted to Harry Hoogstraal, Head of the Department of Medical Zoology, Naval Medical Research Unit No. 3, who gave assistance in obtaining the many wild rodents used. He also has provided the authors with the host identifications. We also wish to thank LT(j.g.) W. H. Wells of NAMRU-3, who took part in the earlier stages of this investigation.

to experimental infection with *S. mansoni* and *S. haematobium** indicated the mammals which should be of primary concern in a search for reservoir hosts. Continued interest in this phase of schistosomiasis led to an expansion of the project to include most of the small species of mammals easily obtainable in Lower Egypt.

MATERIALS AND METHODS

Most of the small species of mammals common to Lower Egypt, and especially the vicinity of Cairo, were represented in the list of animals exposed to counted numbers of cercariae of *S. mansoni*. Hosts employed were:

Mus musculus praetextus Brants—the common house mouse, found in human habitations throughout Egypt.

Rattus rattus (L.)—the common house rat, found throughout Egypt.

Arvicanthis niloticus (Desmarest)—the Nile rat, a rodent very common in the cultivated areas of Egypt; frequently found along canals and road banks in the vicinity of villages in the rural districts.

Acomys cahirinus (Desmarest)—the spiny-back mouse, found in the habitations of man as well as in the fields.

Gerbillus pyramidum Geoffroy—the gerbil or common desert mouse, found commonly in the desert, especially in areas adjacent to cultivated lands.

Jaculus jaculus L.—the gerboa or desert jumping mouse, found in desert areas with sparse or moderate vegetation.

Meriones s. shawi Duvernoy—the jird, a rodent found in the Eastern Desert of Egypt.

Psammomys o. obesus Cretzschmar—the fat sand rat, found commonly in the semi-desert areas of the Mediterranean littoral plain.

Nesokia indica suilla Thomas—the Egyptian bandicoot, found in the cultivated areas adjacent to the desert in the Lake Qarun area of Fayeum Province.

Hemiechinus auritus aegyptius Fischer—the Egyptian hedgehog, an insectivore found frequently in gardens and villages throughout Lower Egypt.

Mustela nivalis subpalmata (Hemprich and Ehrenberg)—the Egyptian weasel, a carnivore common in villages and cities.

Herpestes i. ichneumon L.—the Egyptian mongoose, inhabits canal banks and cultivated areas throughout the irrigated sections of Egypt.

Vulpes v. aegyptiaca Sonnini—the common Egyptian fox, frequents the cultivated as well as the marginal desert areas.

When feasible, laboratory-raised hosts were employed to avoid the use of animals possibly infected in nature. *Mus*, *Rattus*, *Arvicanthis*, *Acomys* and *Meriones* reproduced in captivity, or in many instances were brought to the laboratory at the weaning age accompanying the mother. The desert rodents are more difficult to maintain and stand practically no chance of being infected naturally, so half grown *Gerbillus*, *Jaculus* and *Psammomys* were selected from

* A paper giving the susceptibility of mammals to infection by *Schistosoma haematobium* is in preparation.

large numbers of stock brought in by Bedouin collectors. It was also necessary to purchase young mongooses, foxes and half grown bandicoots and hedgehogs. The weasels were trapped in homes and public buildings in Cairo.

The smaller rodents were exposed to 200 cercariae each since previous work (Stirewalt *et al.*, 1951) indicated this number to be an optimal exposure density. Also, the data obtained from these infections allowed for comparison with similar investigations relating to the susceptibility of laboratory animals to infection by *S. mansoni* of Egyptian origin (Kuntz *et al.*, 1955). Five hundred ml. capacity crystallizing dishes with one half an inch of pond water containing the cercarial suspension were used for the smaller rodents, following a preliminary immersion of 15 minutes in warm water to encourage the passage of urine and feces. Dishes of one liter or greater capacity were used for the larger hosts. The weasels, due to their extreme agility and ferociousness, were placed in a wire mesh cage which was lowered into a cercarial suspension. Mongooses and foxes were held in a 16 liter capacity glass container with 1,000 to 1,500 cercariae and 2,500 to 5,000 cercariae respectively.

Cercariae were obtained from 20 to 30 naturally infected *Biomphalaria boissyi* and counted as each drop of cercarial suspension was placed on the bottom of the exposure containers. Numbers of cercariae were estimated for exposure of the larger animals. Only the immersion method has been used since it more nearly duplicates the mode of infection in nature. All hosts were exposed at room temperatures for a period of 30 to 45 minutes.

Hosts were autopsied at death or at various intervals (1949 to 1952) after a period of time (6–14 weeks) sufficient to allow the parasites to reach maturity. Some hosts were allowed to live longer to determine whether duration of infection would influence the parasite returns, location of schistosomes in the host, egg deposition, etc. At the time of autopsy hosts were killed with chloroform and the abdominal and thoracic viscera were removed *en masse*. Organs were examined separately with the aid of a dissecting microscope. All tissues were teased apart with splinter forceps to reveal the presence of parasites. After preliminary examination and removal of parasites the macerated tissues were shaken in a stoppered flask in several changes of physiological saline and re-examined to recover any remaining schistosomes.

Intact parasites were used for measurement purposes after standing in physiological saline for four to six hours. After this period of semi-relaxation worms transferred to microslides were easily measured with the aid of a dissecting microscope and a ruler placed beneath the slide. Splenic enlargement was judged by comparing this organ from infected animals with the spleens removed from eight or ten control animals which were not infected.

RESULTS AND DISCUSSION

It has been known since the earlier investigations on Oriental schistosomiasis that many domestic and wild mammals are readily susceptible to infection by *S. japonicum* and that such animals may even serve as reservoir hosts. Studies on the susceptibility of animals to infection by the other schistosomes of man,

i.e. *S. mansoni* and *S. haematobium*, have progressed more slowly and emphasis has been primarily on *S. mansoni* because of the availability of materials to research activities. Until recently (Schwetz, 1953) little attention has been given to the infection of wild mammals to infection by *S. mansoni* or to a search for reservoir hosts in endemic areas. The present paper gives not only the relative susceptibilities of a number of wild mammals to infection but also indicates those hosts that may be employed in experimental schistosomiasis and supports the contention that wild rodents could possibly be infected naturally should the proper circumstances prevail.

Findings in this study may be easily compared with previous data giving the relative susceptibilities of laboratory animals to infection with *S. mansoni* since the same procedures were followed in infection, removal of parasites and in recording the data. Pertinent information including the hosts employed, duration of infections, parasite recoveries, deaths among hosts infected and the distribution of parasites and their eggs in organs is given in Tables 1 and 2. Data on certain hosts such as foxes, mongooses, weasels and the fat sand rat (*Psammomys*) are not included in the tables since the circumstances relating to their exposures and maturation of infections are somewhat different and the data thus obtained are not suitable for comparison.

Although all hosts, with the exception of the foxes, exposed to schistosome cercariae showed evidence of parasite invasion, there is considerable latitude in the susceptibility of the host species, as well as among the individuals of the species, to infection by *S. mansoni*. Pathological conditions resulting from infections show wide ranges of variability even in those hosts which are considered satisfactory for the parasite. The production of, and the passage of viable eggs may follow an erratic course and the deposition of eggs in the organs is dependent in part upon the location of the mature parasites in the host as well as upon the tolerance of the host for the parasite. The age factor was not constant in this investigation although an attempt was made, when feasible, to select hosts of medium size or those just nearing maturity.

Mus musculus. Although the recovery of schistosome parasites was somewhat less, the general conditions attendant to infection were similar to those recorded for the albino mouse (*Mus musculus domesticus*) (Kuntz *et al.*, 1955) employed in an earlier study and infected with the *S. mansoni* of Egyptian origin. There is a definite tendency toward elimination of parasites as the duration of infections is prolonged. In both the house and albino mice the recovery of worms was reduced to approximately 3.0 per cent in hosts (Table 1, a) living for 85 to 119 weeks after infection. Although there was an absence of worms at the time of autopsy in a few mice, there were remains of recently dead worms and eggs in the liver and parts of the intestine of all hosts. Viable eggs though few in number may persist in the tissues for several weeks beyond the time when any remains of parasites may be detected.

There was a predominance of male schistosomes from the house mice in the three age categories and the measurements of both sexes were near those given for parasites from the albino mice. Although some of the worms were quite small,

TABLE 1

Parasites recovered from wild mammals exposed to 200 cercariae of Schistosoma mansoni *in Egypt*

Duration of Infection	Number of Hosts Examined	Deaths Among Hosts Exposed (Percentage)	Worms Recovered at Autopsy		Sex of Worms (Percentage)		Worm Measurements (Mean) in Millimeters	
			Range	Percentage	Males	Females	Males	Females
A. RODENTS								
a. *Mus musculus*—House Mice								
8–14	20	25*	4–67	14.8	61*	39	8.0	10.8
16–48	9	44	4 25	9.4	54	46	12.1	15.6
52–119	6	83	0†–16	3.5	56	44	12.4	16.8
b. *Rattus rattus*—House Rats								
8–10	19	5.0	2–50	7.7	56	44	6.1	8.4
11–19	8	0	1–30	1.8	59	41	7.8	9.6
20–27	6‡	0	0†	0‡	0	0	0	0
c. *Arvicanthis niloticus*—Nile Rats								
8–14	24	17	4–124	18.1	52	48	11.8	14.8
16–26	15	13	1–61	11.2	60	40	13.2	15.1
53–145	10	60	1–56	8.8	53	47	14.4	16.8
d. *Acomys cahirinus*—Spiny Back Mice								
8–12	27	15	13–85	16.6	58	42	8.0	11.4
14–74	10	20	3–45	7.2	51	49	11.1	14.1
e. *Gerbillus pyramidum*—Gerbils								
6–11	37	81	12–80	18.8	54	46	9.6	12.2
18–21	5	0	3–12	4.1	44	56	—	—
104–112	2	100	0†	0†	—	—	—	—
f. *Jaculus jaculus*—Gerboas								
6–11	22	82	3–46	9.6	52	48	7.7	10.5
g. *Meriones shawi*—Jirds								
11–17	20	5	4–82	13.5	61	39	11.6	13.6
30–34	6	17	10–16	7.4	46	54	13.4	15.7
h. *Nesokia indica*—Bandicoots								
3–21	7	29	14–64	17.2	28	72	11.3	11.9
B. INSECTIVORE								
i. *Hemiechinus auritus*—Hedgehogs								
10–14	6	33	0†–15	4.3	70	30	10.1	11.2

* Nearest whole numbers used when feasible.

† Hosts though negative for parasites showed definite evidence of earlier infection.

‡ All hosts exposed to same lot of cercariae: evidence of infection in 4/6 hosts though no worms were present.

TABLE 2

Distribution of parasites and their eggs in wild mammals exposed to 200 cercariae of Schistosoma mansoni *in Egypt*

Duration of Infection (Weeks)	Number of Hosts Examined	Location of Parasites in Hosts; Total No. (Percentage) of Worms in Veins of:				Hosts (Percentage) with Eggs in tissues				Hosts (Percentage) Showing Spleno-megaly§
		Liver†	S. & L. int‡	Lungs	Spleen	Liver	S. & L. int	Lungs	Spleen	
A. RODENTS										
a. *Mus musculus*—House Mice										
8–14	20	63*	36	0	0.8	90	85	0	0	15
16–48	9	71	28	0.3	0.2	89	100	0	11	22
52–119	6	65	34	0.5	0	17	100	17	17	17
b. *Rattus rattus*—House Rats										
8–11	19	94	5	0.9	0	47	26	0	0	0
12–19	8	96	4	0	0	62	37	0	0	0
20–27	6	0¶	0	0	0	50	33	0	0	0
c. *Arvicanthis niloticus*—Nile Rats										
8–14	24	91	8	0	0.9	71	71	0	0	4
16–26	15	56	41	2	0.7	100	93	20	13	20
53–145	10	29	62	7	2	90	100	30	20	50
d. *Acomys cahirinus*—Spiny Back Mice										
8–12	27	81	16	2	0.8	74	85	0	7	17
14–74	10	66	33	0	0.6	90	100	0	10	20
e. *Gerbillus pyramidum*—Gerbils										
6–11	37	61	38	0	1	81	89	0	8	8
18–21	5	70	28	0	2	100	80	0	20	20
104–112	2	0¶	0	0	0	100	100	0	50	50
f. *Jaculus jaculus*—Gerboas										
6–11	22	57	43	0	0	36	59	0	0	0
g. *Meriones shawi*—Jirds										
11–17	20	68	32	0	0	100	100	0	2	45
30–34	6	3	97	0	0	100	100	0	17	50
h. *Nesokia indica*—Bandicoots										
8–21	7	85	13	0	2	100	86	0	0	0
B. INSECTIVORE										
i. *Hemiechinus auritus*—Hedgehogs										
10–14	6	79	17	0	4	33	50	0	0	0

* Nearest whole numbers used when feasible.

† Liver and hepatic-portal system.

‡ Mesenteric veins of small and large intestine.

§ Splenic enlargement judged by comparison with spleen size in noninfected controls

¶ Hosts though negative for parasites showed definite evidence of earlier infection.

the average lengths of parasites were greater in those hosts which lived beyond two years. There appeared to be no constant relationship in worm size and the location in the host, indicating that the parasites probably migrate from vessels in the mesenteric circulation to the hepatic-portal vessels and back. It should be noted however that greater numbers of eggs appeared in the more posterior parts of the intestinal tract in those hosts with a larger number of worms residing in the mesenteric system. As a rule eggs appeared first in the liver, then in the beginning of the small intestine. In older infections eggs were found throughout the entire length of the small intestine and frequently in the large intestine. In a few individuals the eggs were numerous in the walls of the rectum and extended into the walls around the anal sphincter. The passage of eggs with the feces seems to follow no fixed pattern. Few to moderate numbers of eggs appeared 8 to 11 weeks after infection. Although the number fluctuates eggs may be passed until death of the host. However there is a tendency toward reduction in quantity of eggs as evidenced in fecal counts made from mice in the old age infection group.

Occasionally a pair of adult worms was found in the renal or splenic veins. In these hosts nests of eggs were found in the vicinity of the worms or in tissues of the organ associated with the vessel in which parasites were found. The occurrence of schistosomes in the lungs is infrequent and is considered as accidental. Adult worms deposit eggs in the tissues of the lungs; however eggs are frequently present in the lungs in the absence of worms.

Ulceration of the intestinal wall with passage of large quantities of blood is not infrequent and appears to be related to the degree of egg deposition in the tissues at a given location. Loss of blood and passage of semi-fluid stools account for a number of deaths. Over two thirds of the infected mice died 16 to 24 weeks after the beginning of infection. Higher death rates in animals which lived beyond the one year period are due in part, perhaps, to age or to a combination of age plus their infection. Some of the surviving hosts had apparently withstood earlier pathological crises as was indicated by extensive areas of tissue damage in hosts that were killed for examination and the subsequent tissue repair observed in hosts of the same group which were allowed to live longer.

Both splenomegaly and hepatomegaly are detectable in infected mice. The latter is more difficult to evaluate since the size of the liver seems to vary even in normal or non-infected mice. Several mice of the 16 to 48 weeks age group possessed livers estimated to be 1½ times normal size. Splenomegaly though marked in a few infected animals cannot be considered as a constant or characteristic feature in schistosome infection in these lower mammals. Frequently the spleen is only slightly enlarged. In two mice, however, the spleen was estimated to be 2½ to 3½ times normal size. On the other hand in several mice with infections of two years duration the spleens were actually atrophied.

The general pathological conditions prevailing in the house mice are similar to those already described in more detail in the albino mouse (Stirewalt *et al.*, 1951; Kuntz *et al.*, 1955). Liver damage ranges from small areas of sclerosis along the borders to those cases in which the entire organ is involved. In old infections the liver may be fibrotic throughout, discolored by localized or overall deposits

of pigment, or occasionally pale to gray due to exsanguination. Some livers are characterized by rough, sandy and hardened surfaces due to the presence of large numbers of eggs in the outermost tissues. Sections of, or the entire small or large intestine may be characterized by nodules, fibrotic areas, patches of diffuse or concentrated pigmentation and with various stages of pathological involvement inside or outside the intestinal wall. Hemorrhages are readily apparent in the intestinal walls and occasionally in the mesenteric tissues. Only a few eggs appear on the walls of the cecum and rarely on the lower wall of the stomach. Involvement of the spleen and kidneys is similar to that seen in the liver but usually the areas of tissue damage and egg deposition are localized and show varying degrees of pigmentation.

The house mouse is considered a favorable host for infection by *S. mansoni*, the infection following the course so well known for its laboratory cousin, the albino mouse.

Rattus rattus. As might be expected after a comparison of infection patterns in wild and albino mice, the same is true generally in the susceptibility of wild and albino rats to infection by *S. mansoni*. In the wild rat, as in its domesticated counterpart, the yield of worms is low, there are no critical pathological manifestations and the infection is self limiting. Parasites may disappear entirely 20 weeks (Table 1, b) after entry into the body, leaving only a few nonviable eggs in the liver or intestinal walls as evidence of earlier infection. Although some of the schistosomes do reach maturity in the rat only a few eggs are produced and deposited in the host's tissues. Only a few viable and nonviable eggs were passed with the feces.

Arvicanthis niloticus. Of the animals evaluated in this study the Nile rat is considered as one of the best hosts for *S. mansoni* infection, since it gives good returns of well developed parasites which produce an abundance of eggs and the subsequent pathological picture typical in experimental schistosomiasis. Parasitological considerations are even better than those portrayed in the mouse since the worms are more widely distributed in the body (Table 2, c). Worms and their eggs were found in the splenic and renal circulation and in the lungs. All parts of the intestinal tract below the stomach were involved suggesting a favorable host-parasite relationship as seen in human infections. There is a tendency toward parasite reduction in prolonged infections (Table 1, c). However, after 2½ years the hosts still maintain a fair proportion of their parasite load (8.8 per cent) and continue to pass viable eggs with the feces until death. Hepatomegaly was observed in a number of hosts and half of the Nile rats in the older age category demonstrated definite splenomegaly. The abdomens of several of the animals which had been infected for 30 weeks or longer were distended due to accumulations of fluid in the body cavity.

A number of *Arvicanthis* exposed to 500 cercariae produced correspondingly larger numbers of parasites and the deposition of more eggs was responsible for greater pathological damage to the hosts. Consequently the death rate was higher and the deaths occurred at an earlier age than in the Nile rats exposed to only 200 cercariae. Only two or three hosts lived beyond 20 weeks. Considerable

quantities of blood and numerous eggs were found in the intestinal contents of animals dying 12 to 16 weeks after infection.

The larger size (equal to, or slightly larger than the albino rat) of a favorable host such as the Nile rat seems to enhance the welfare of the schistosome parasites in allowing freedom of movement to optimal locations within the host, a limitation which in the mouse may be due to physical barriers such as the small caliber of vessels in the venous system. Furthermore the passage of considerable numbers of eggs seems to be related to the greater number of parasites which are found in the lower and rectal sections of the large intestine in *Arvicanthis*. Eggs in the walls of the Nile rat give rise to open lesions in the rectum, a condition not observed in other small hosts. Eggs could easily be removed from rectal scrapings of the living animals. This condition offers definite advantages for specific types of investigation in experimental schistosomiasis.

Passage of numbers of eggs by *Arvicanthis* allowed opportunity for additional observations. A collection of pellets taken over 24 hour periods indicated that more eggs were passed during the early morning hours than during the later daylight hours. This finding is to be expected since the wild hosts were very active and consumed greater quantities of food during the hours of darkness. The accelerated intestinal activity plus the passage of greater quantities of feces results in release of more eggs from the rectal walls. This phenomenon parallels that observed in man in which case the eggs of *S. haematobium* are passed in the urine in much greater numbers following strenuous exercise.

Acomys cahirinus. The spiny-back mouse is readily infected with *S. mansoni* and maintains its infection with a moderate parasite load for long periods but the small size of the host reduces the chance for attaining the rather normal host-parasite relationship witnessed in *Arvicanthis*. The infections follow the course described for wild and albino mice. The pathological findings are similar and the pattern of egg deposition and passage with feces is approximately the same. *Acomys* is a rodent with a very delicate skin which is subject to easy injury, consequently obvious difficulties arise in handling this host in the laboratory.

Gerbillus pyramidum. The gerbil is quite susceptible to schistosome infection, giving a rather high return of parasites which deposit numbers of eggs in the host tissues. Moderate quantities of eggs are passed with the feces from the 10th week onward until death. The gerbil however ranks second to the house mouse in this respect. This desert rodent is undesirable for experimental work because of the high death rate among animals two to four days after exposure and then again, during the early periods of maturation of the infection. Many of deaths soon after exposure are probably due to pneumonia. Apparently there is an active host protection phenomenon which tends to reduce the number of parasites (4.1 per cent, Table 1, e) by the time the infection has become 18 to 21 weeks of age. As with *Rattus*, the worms may disappear entirely by the end of two years leaving only repaired tissue and deposits of eggs as evidence of earlier infection.

Jaculus jaculus. This species of jerboa is unsatisfactory for use with experimental schistosomiasis since it does not readily adapt to laboratory conditions and there are numerous deaths a day or so after exposure, as well as at later

intervals in the maturing infections. In the earlier stages of infection there was a return of only 9.6 per cent (Table 1, f) of the parasites and the jerboas did not live beyond the 11th week. Only a few eggs were found in the tissues of the liver and the intestine. It seems unlikely that these hosts died from the schistosome infection alone.

The host protective mechanism as indicated by obvious reductions in parasite load, is more pronounced in *Jaculus jaculus* than in *Gerbillus* thus classifying the former as a poor host for *S. mansoni* infection.

Psammomys obesus. This desert rodent is not listed in the tables since none of the 11 hosts exposed to cercarial suspensions lived more than four to six days. Hemorrhages in the lungs indicated that the schistosomes had penetrated the hosts and had gained entry to the circulatory system. Presumably, deaths were not due to schistosome infection.

Meriones shawi. The jerd, though inferior to the house mouse and the Nile rat, is considered as an acceptable host for experimental schistosomiasis mansoni. Although the return of worms, i.e., 13.5 to 7.4 per cent (Table 1, g), is not as great as in some hosts, the worms recovered are well developed and are capable of depositing an abundance of eggs in the liver and throughout the intestine. Deaths among the groups of *Meriones* exposed to *S. mansoni* were not as great as with the other desert species employed. The survival rate of hosts living one week beyond infection date is high. The reduction of parasite burden after 30 weeks indicates a host protective mechanism which in efficiency is intermediate between *Mus* and *Arvicanthis.* In one of six hosts the spleen was three times normal. *Meriones* passes appreciable numbers of viable eggs in its fecal pellets. This host adapts readily to laboratory conditions and handling, and breeds quite well in captivity.

Nesokia indica. The so-called bandicoot is very susceptible to infection giving a high return (17.2 per cent, Table 1, h) of moderately large parasites which deposit numerous eggs in the liver and parts of the intestine. It is of interest to note the prevalence (72 per cent) of female schistosomes recovered from *Nesokia* since male worms have been in the majority in most categories of animals considered. Although the bandicoot passes a fair number of eggs with the feces, it does not exhibit the critical pathological conditions observed in mice and in the Nile rats. The larger size of this host (1 to 1½ times the size of the albino rat) does allow appreciable movement of worms to, and deposits of eggs in all parts of the intestine posterior to the stomach.

The bandicoot is not classed as an ideal laboratory host because of its unpredictable disposition and the general difficulties entailed in handling it in captivity. It does not breed in the laboratory and is not always obtainable in large numbers since it is somewhat restricted in its distribution.

Hemiechinus auritus. The hedgehog was chosen for evaluation in this study because of its availability and the fact that it represents a different order of mammals. This insectivore produced only a few (4.3 per cent, Table 1, i) moderately well developed worms which deposited only limited quantities of eggs in the liver and intestine. The majority of eggs examined from tissues were

nonviable. Although there were nests of eggs in the walls of the lower part of the intestine the serious pathological reaction was not present as seen in the more favorable hosts. Only a few of the female schistosomes possessed eggs in their uteri.

Obviously the compatibility of host and parasites in this mammal is only fair. As a matter of fact one host had lost all of its worms by the end of the 11th week.

Mustela nivalis. The weasel, a member of the Carnivora, is one of the most difficult of animals to handle. Although the weasels were given special attention and care, none of the six animals lived for more than five days after exposure. Only one of the five showed evidence of parasite invasion. The one weasel had small scattered hemorrhages in the lungs indicating that some of the parasites may have gained entry into the host's circulatory system. Death of the weasels was attributed to pneumonia.

The weasel, as other carnivores, seems to offer a definite resistance to infection by *S. mansoni*, the host-parasite relationship verging on incompatibility.

Herpestes ichneumon. Only two young mongooses were exposed to infection. Contrary to the schedule followed with rodents, the mongooses were exposed to 1,000 to 1,500 cercariae. Twelve weeks later 17 schistosomes were removed from one mongoose and 46 from the other. The majority of worms were found in the vessels of the lower part of the small intestine. The parasites though of moderate size apparently produced no viable eggs. Only shells and degenerating eggs were found in the liver and the walls of the small intestine.

Such a small return of worms with limited production of nonviable eggs classifies the mongoose as a host with unusually low tolerance for schistosome parasites, a characteristic common to the representatives of the Carnivora thus far considered.

Vulpes. Three young foxes were exposed to 2,500 to 5,000 cercariae each by the usual partial immersion method. No parasites or evidence of entry of parasites into the host were detected when the foxes were examined 6 to 10 weeks later. Thus the fox is considered as a nonsusceptible, or as incompatible to infection with *S. mansoni*. This condition agrees in general with the results described for canines in an earlier susceptibility study (Kuntz *et al.*, 1955) in this laboratory in which only a few schistosomes in retarded stages of development were found in the liver of one of 11 dogs after exposure to 6,000 to 8,000 cercariae.

Previous studies in this laboratory plus the present data based on the exposure of quite a series of wild mammals to infection by *S. mansoni*, and the subsequent recovery of worms indicate that all rodents probably are susceptible to infection by this parasite. The carnivores as a group are very poor definitive hosts and most of them, including the mongoose, are considered as only slightly susceptible to infection. Apparently some of the schistosomes enter the circulatory system but soon succumb to the host's ability to destroy the parasites. A small percentage of only moderately well developed schistosomes survive in the domestic cat (Kuntz *et al.*, 1955) and these do not produce many eggs or the subsequent pathological conditions characteristic of infections in most of the rodents. Rarely a few extremely small and poorly developed schistosomes may be recovered from

the liver of dogs (Kuntz *et al.*, 1955). The hedgehog, an insectivore, is also a poor host which may be placed intermediate in the scale of susceptibility between the rodents and the carnivores.

In general the conditions of schistosome infection in small wild animals parallel those already described for laboratory hosts (Stirewalt, *et al.*, 1951; Kuntz *et al.*, 1955). Our data provide interesting comparisons of the schistosome infection pattern in the wild mouse and rat, as contrasted with their counterparts the albino laboratory mouse and rat. The recovery of parasites from the wild *Mus* are lower than for the laboratory mouse in the earlier stages of infection, i.e., in the range of 10 to 40 weeks. However, the tendency toward parasite elimination is approximately the same (3.5 and 3.2 per cent, respectively) in the mice examined 40 to 119 weeks after infection. In both groups two or three individuals had lost all of their parasites a year or more after exposure but in each case there was obvious evidence of earlier infection. The yield of schistosomes from wild *Rattus* was slightly greater than from the laboratory rat but with both hosts there was a marked reduction in numbers of worms with prolongation of periods of infection and there was an absence of parasites in some individuals. As a matter of fact no parasites were found in six wild *Rattus* which were examined 20 to 27 weeks after exposure (Table 1, b). Although our observations do not indicate a precise relationship between the host's tolerance for schistosome infection and egg production by mature parasites, there is a suggestion that female worms in the more favorable hosts do pass greater quantities of eggs. Eggs are produced by some but not all the schistosomes in unfavorable hosts such as the wild and laboratory rat and the hedgehog. In these cases the majority of eggs are usually found in the liver of the host and consequently only a very few, if any, are passed with the feces. Therefore the failure to recover eggs from the host's feces is, in part, an indication of an unsatisfactory host-parasite relationship. Apparently an unsatisfactory host-parasite relationship does not apply to all worms within the host, since in *Rattus*, a poor host, only one or two among 8 or 10 females may possess well developed eggs in the uterus. Deposits of eggs in tissues are frequently associated with an egg producing female whereas other females without an egg in the uterus may be found in a vessel where no eggs have been deposited. Conversely, in mice or *Arvicanthis* which are considered as favorable hosts, most of the female schistosomes possess an egg in the uterus but a few individuals possess none. Occasionally the latter females are found in a vessel or tissue containing no eggs, further supporting the belief that some of the females may be sterile or pass through periods when no eggs are produced.

An examination of several hundred animals in our susceptibility studies shows that paired schistosomes produce eggs although the worms have been found in unusual locations in the host. Schistosomes and their deposits of eggs have been found in the spleen, the kidneys and in the lungs. On several occasions moderately well developed parasites in the lungs were surrounded by tissues impregnated with hundreds of eggs. Although the mesenteric and hepatic-portal parts of the venous systems seem to be the more ideal sites for the schistosome, the parasite can live quite normally in other parts of the body. This may account for the occasional finding of schistosome eggs at seemingly bizarre sites in man.

Observations in this investigation supplement other reports and descriptions of conditions recorded in previous susceptibility studies: (1) splenomegaly does occur in wild, as well as in small laboratory mammals infected experimentally with *S. mansoni*. Our data do not give conclusive information indicating an exact relationship of incidence and degree of splenic enlargement as related to parasite loads or duration of infection. (2) Measurements of parasites from both favorable and only fair hosts cover quite a range, thus supporting our previous contention that size alone cannot be employed as a criterion for judging the normality of worms. (3) Our data further indicate that there is a natural predominance of male parasites. Male worms were in the majority in most categories of hosts examined. A notable exception was in the bandicoot in which 72 per cent of the worms recovered were females. (4) Furthermore this study, in agreement with similar work by others (Evans and Stirewalt, 1951) indicates that the recovery of worms from hosts varies with the different batches of cercariae for infection purposes.

Approximately 500 rodents and other small mammals from areas with a known high incidence of intestinal schistosomiasis were examined following the preliminary evaluation of host susceptibilities in this work. In a search for reservoir hosts emphasis was placed on the capture and examination of the Nile rat, since it proved to be such a satisfactory host and its natural habitat is such as to make infection in nature quite possible. Although approximately 100 *Arvicanthis* were taken along the canals in localities where *S. mansoni* infection is common in the population (40 to 80 per cent) none of the rats were found infected. This was somewhat of a surprise since *Arvicanthis* had been observed feeding and drinking at the edge of infected canals and on occasion were seen swimming in the water. The survey was not entirely fruitless, however, since one *Gerbillus pyramidum* was found infected (Kuntz, 1952) with *S. mansoni*. In this case the infection was considered as accidental, resulting possibly from the host eating an infected snail which had been stranded above the usual water level following the annual Nile flood.

With the continued interest and investigation in the susceptibility phases of schistosomiasis plus information from surveys of mammals in areas endemic for intestinal schistosomiasis (Schwetz, 1953), it has become apparent that *S. mansoni* shows little specificity for its definitive hosts. It is now known that animals from quite a number of representative groups of the Mammalia may be infected with *S. mansoni*: the hairy armadillo, *Euphractus sexcinctus* (Pinto, 1944); the crab eating raccoon, *Procyon cancrivorus* (Ruiz, 1952); the rice rat *Oryzomys palustris* (Moore and Meleney, 1952); and the agouti, *Dasyprocta aguti* (Price, 1953). The Syrian hamster, *Cricetus auratus*, and the cotton rat, *Sigmodon hispidus*, should be included since they represent wild rodents recently adapted as laboratory animals. Of special note is the recent report by Barbosa *et al.* (1953) describing the discovery of a number of *Rattus r. frugivorus* naturally infected with *S. mansoni* in Brazil, and the description of a variety of *S. mansoni* (*Schistosoma mansoni* var. *rodentorum*) (Schwetz, 1953) from the rodents, *Mastomys coucha*, *Pelomys fallax*, *Dasymys bentleyae* and *Lophuromys aquila* in the Belgian Congo. Our study adds eight species of rodents, one carnivore and an

insectivore to the list. The number of hosts known to be susceptible to *S. mansoni* infection is increased if one includes the primates (Kagan, 1953; Amberson and Schwarz, 1953).

As might be expected with our present knowledge of the susceptibilities of various animals to helminth infections, all hosts, including those demonstrating favorable compatibility or host-parasite relationships, show a considerable range of parasite tolerance. The accumulation of data elucidating the various aspects or phases in the biology of the schistosomes reveals the many intricacies upon which the basic condition of successful animal parasitism may depend. As the interest in this general field of investigation expands it is realized that many factors including physiological, genetic and geographical relationships of parasite to snail and parasite to definitive host must be considered. Additional problems, including the possibility of reservoir hosts for *S. mansoni*, become evident as increasing numbers of hosts are evaluated for their abilities to maintain or to protect themselves against schistosome infections.

SUMMARY

Nine species of wild rodents, three carnivores and an insectivore from Lower Egypt have been subjected to experimental infection by *Schistosoma mansoni*. The rodents, as a group, are the most acceptable hosts for *S. mansoni*, whereas the carnivores at their best show only negligible susceptibility to infection. The hedgehog, an insectivore, is a poor host occupying an intermediate position between the rodents and the carnivores.

As expected, the recovery of schistosomes and the conditions attendant to infection in all hosts, including the species with the most favorable host-parasite relationships, show a considerable range of variability. Egg production by the parasites is also quite variable. Schistosomes in poor hosts tend to produce fewer eggs than parasites in satisfactory hosts. Apparently the relationship between egg production and host-parasite compatibility is not absolute and does not apply to the individual worms. Some individuals of a group of schistosomes in a poor host produce eggs, while others do not. Furthermore, the degree and incidence of splenic enlargement in small mammals infected with *S. mansoni* follows no definite pattern in the various hosts examined.

Arvicanthis niloticus, the Nile rat, is the best of the rodents studied from the standpoint of general parasitological relationships and requirements, and gives satisfactory yields of well-developed schistosomes which deposit numerous eggs in the wall of the lower intestine as well as in other organs of the body. *Arvicanthis* has not yet been found naturally infected although it is a satisfactory host for *S. mansoni* and is frequently found near or even in the water of irrigation systems in areas where schistosome infection is common in snails and in man.

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STUDIES ON MITES AS VECTORS OF WESTERN EQUINE AND ST. LOUIS ENCEPHALITIS VIRUSES IN CALIFORNIA[1]

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The actual role of mites as vectors of the arthropod-borne viral encephalitides has been of considerable interest to epidemiologists who study these infections. Proof that mosquitoes are natural vectors of the viruses of this group left the following epidemiological aspects still unknown: the means of winter carry-over of infection in endemic areas and the periodic appearance and disappearance of infection in certain areas. In the widening search for other arthropod vectors and reservoirs, a number of research groups carried out intensive investigations of bloodsucking mites parasitic on domestic and wild birds. As a result, these viruses were isolated numerous times from a variety of mites (Smith *et al.*, 1944, 1945; Sulkin, 1945; Sulkin and Izumi, 1947; Reeves, Hammon *et al.*, 1947; Hammon, Reeves *et al.*, 1948; Howitt *et al.*, 1948; Miles *et al.*, 1951), and one research group (Smith *et al.*, 1946, 1947, 1948) reported successful experimental transmissions and even transovarian passage of the St. Louis virus by the mite *Dermanyssus gallinae*. Following these reports, mites have been accepted as vectors to avian hosts and reservoirs of the entire group of arthropod-borne viral encephalitides by a large number of scientific workers and this is frequently pointed out in medical journals and textbooks.

Some research workers in this field have felt that this subject deserved additional extensive and critical study. At least five groups have undertaken field and laboratory investigations, and the conclusions of three of them are the subject of the present series of papers (Chamberlain and Sikes, 1955; Sulkin *et al.*, 1955; and the present paper). The data to be presented leave considerable doubt as to the *widespread importance* of the species of bloodsucking mites studied as vectors or reservoirs of the encephalitis viruses.

The present paper describes the field and laboratory experience of the Encephalitis Research Unit of the Hooper Foundation over a period of nine years (1944–1952). The principal purpose of the field investigations was to determine whether encephalitis viruses could be found in naturally infected mites in Kern County, California, an area in which Western equine encephalomyelitis (W.E.E.)

[1] This investigation was supported in part by a research grant G-481 (C6) from the National Microbiological Institute of the National Institutes of Health, Public Health Service, and in part under the sponsorship of the Commission on Virus and Rickettsial Diseases, Armed Forced Epidemiological Board, Office of the Surgeon General, Department of the Army.

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and St. Louis encephalitis (S.L.E.) viruses are active at a highly endemic level each summer. The purpose of the laboratory studies was to test further the ability of *D. gallinae* and *Bdellonyssus sylviarum* to serve as vectors or hosts of W.E.E. and S.L.E. viruses.

METHODS

Field investigations

Collections. Mites were collected from locations where mosquitoes had been found infected in several preceding summers. From 1946 through 1949 wild bird nests were kept under observation. As the peak populations of the parasitic mites in question were associated primarily with nestlings, nests—usually those in which mites were observed—were collected just before or immediately after the young birds left the nest, and were taken to the field laboratory. Mites for virus tests were aspirated into a modified Pasteur pipette. The usual minimal number of mites collected in each pipette was 100 and the maximum 250. An arbitrary maximal sample size was set at four tubes of 250 specimens, or 1,000 mites from each nest; only occasionally were larger samples taken. Undoubtedly, some counts were too low because it was difficult to suck only one mite into the tube at a time from heavily infested nests. Each pipette containing a collection of mites was sealed at both ends in a gas flame, and frozen in dry ice.

For taxonomy a sample of mites equaling at least 10 per cent of the number in the frozen sample was collected from each nest and preserved in 70 per cent alcohol.

Virus testing. Each tube was opened and the frozen mites it contained were transferred to a chilled mortar and ground with alundum in 3 ml. of 30 per cent rabbit serum broth. The suspension was centrifuged for 10 minutes at 3,000 r.p.m. and the supernatant fluid was removed for inoculation. In 1946 and 1947 the supernatant fluid was inoculated into five or six baby mice four days old, each being inoculated with 0.03 ml. by the intraperitoneal route. An additional 0.8 ml. of the supernatant fluid was added to 0.4 ml. of a 1:2,500 dilution of phenyl mercuric borate and 0.4 ml. of penicillin solution containing 2,500 units per ml. This suspension was held overnight at 38° to 40°F. and then inoculated into five 21-day-old mice, each mouse inoculated with 0.03 ml. by the intracerebral route. The mice were observed for signs of encephalitis for 21 days after inoculation. Only rarely did supernatant fluids contain enough bacteria to affect the animals, and this occurrence was further reduced by a change in technique in 1948. This technique was to add to 1 ml. of the supernatant fluid of the triturated mites an equal volume of a saline solution containing 1,250 units of penicillin and 250 mg. of streptomycin. The mixture was kept in the refrigerator 45 minutes to 1 hour prior to inoculation. The test animals received the following inoculations: 21-day-old mice, 0.03 ml. intracerebrally and 0.09 ml. intraperitoneally; 4– to 10-day-old baby mice, 0.03 ml. intraperitoneally; 9-day-old embryonated eggs, 0.05 ml. intra-allantoically.

When encephalitis viruses were isolated, they were identified by neutralization tests in mice with specific W.E.E. and S.L.E. antisera, with a normal rabbit

serum control. Additional identification was made by challenge inoculation of W.E.E.-immune and normal guinea pigs. The one isolate not readily identified by the above procedure has been described (Hammon *et al.*, 1948). Recently, this has been identified as a mixture of W.E.E. and S.L.E. viruses.

Mite identification. The mites preserved in alcohol were mounted permanently on slides. If possible, each specimen was examined, and the species and stage of development were noted. The resulting record was used in determining the approximate numbers and species of mites tested for virus, in identifying probable species included in virus positive samples, and in considering the general biology of the mites.

Laboratory investigations

Dermanyssus gallinae. Three colonies of *D. gallinae* were established in a laboratory insectary with mites collected in poultry houses in Riverside, Petaluma and Walnut Creek, California. The average temperature in the insectary was 82°F. and the relative humidity 70 per cent. Colonies were maintained in earthenware crocks with adequate safeguards to prevent mites from escaping. A platform of hardware cloth was placed over sawdust in the crock bottom and shelter for the mites was provided by pairs of thin boards approximately 2″ × 4″ × ¼″ held together by rubber bands. Chickens provided a source of food for the mites and were confined in the crocks by hardware-cloth covers. Subcolonies were established for individual experiments by transferring a pair of shelter boards from the parent colony to clean crocks containing uninfested birds.

Virus-inoculated chicks, two to six weeks of age, were introduced into the colonies to serve as a source of infection for the mites. The viruses used were S.L.E. strain F 103 and W.E.E. strain F 199, isolated from mosquitoes and having undergone less than ten mouse brain passages; and S.L.E. strain BFS 2131 (third mouse passage) and W.E.E. strain BFS 1034 (fourth mouse passage), isolated from wild bird mites (*B. sylviarum*). The chicks were inoculated subcutaneously with titrated mouse brain suspensions of the viruses, 0.1 or 0.2 ml. containing 10–100 mouse LD_{50}'s, and approximately 24 hours was allowed for development of viremia before the chicks were introduced into the colonies.

Tests for viremia were usually made 24, 48 and 72 hours after inoculation. Blood was mixed with an equal volume of 1:4,000 heparin in sterile water and inoculated intracerebrally into 5 mice, 21 to 28 days old, using 0.03 ml. per mouse. Mice were observed daily for 19 days for evidence of infection. Chicks were removed from the mite colonies after 1 to 7 days and retained in sterilized crocks. These chicks were bled, usually after 3 weeks, and their blood was examined for antibodies. A neutralization index of over 100 was considered positive, 10–99 equivocal, and less than 10 negative.

Samples of mites for virus isolation attempts were obtained from the colonies at various intervals after feeding on inoculated chicks, and were tested by the same procedures as described for field mite samples. The stock colony of mites was presumed to be virus-free because tests of several samples of 250 mites each were negative. In the last experiments an additional virus testing technique was utilized. A sample of approximately 3,000 mites was taken and tri-

turated as above, but 10 ml. of inactivated rabbit serum and 20 ml. of normal saline were used. This suspension was centrifuged for 30 minutes at 16,000 r.p.m. (20,000 g.) to remove debris and reduce the bacterial content. The supernatant fluid was spun in a refrigerated ultracentrifuge for 1 hour at 30,000 r.p.m. (90,000 g.). The pellet was then resuspended in 3 ml. of 30 per cent normal rabbit serum in saline. This suspension, concentrated 10 times by the above procedure, was used to inoculate test animals. In later tests, the inoculum was also treated with the penicillin-streptomycin mixture as an additional safeguard against bacterial contamination.

Normal chicks, 2 to 6 weeks of age, were introduced into the colonies after the mites had an opportunity to feed on inoculated chicks, and remained there from 1 to 5 days. Some were bled 24, 48 and 72 hours after introduction into the colony, and their blood was tested for virus by intracerebral inoculation into 3- to 4-week-old mice. All such exposed chicks were retained and those surviving 3 weeks or longer were tested for antibodies by the virus neutralization test. The interpretation of the neutralization indices was on the same basis as was described above.

Bdellonyssus sylviarum

Dove colony. An infestation of *B. sylviarum* was observed on a colony of hybrid doves maintained in an outdoor screened aviary at the Bakersfield Encephalitis Field Station. Frequently, when a new brood of doves (usually 2 per nest) hatched, these mites would heavily infest the nestlings, reaching a peak population when the birds were about 1 week old. As the nestlings further matured, the mite population in the nest and on its occupants would decrease progressively until by the time the birds could fly it was difficult to find a mite on them. The doves were quite prolific, each pair producing up to 8 broods of young per year. The conditions in this aviary offered promise for study of the ability of *B. sylviarum*, parasitizing an avian host, to serve as a vector and reservoir of the encephalitis viruses at the temperature and humidity prevalent in an endemic area. The most obvious environmental factor excluded was the mosquito vector. Each pair of doves was in a separate cage but these cages were adjoining so that mites could move freely from one unit to another.

On several occasions in the first year of experiments (1949), the population of naturally occurring mites appeared to be very small. On these occasions, additional mites collected from wild bird nests were placed on nestling doves to assist in building up a large population of mites. Such additions were not necessary in 1950, 1951, or 1952.

A possible source of virus infection was introduced by the subcutaneous inoculation of nestlings 6 to 15 days old with 0.2 ml. of 100 LD_{50} mouse dilution of W.E.E. (strain BFS 1034) or S.L.E. (strain BFS 2131) virus.

In 1949, 23 nestlings which hatched during the summer were inoculated with virus. In nests where two young were hatched, one type of virus was given to one nestling and the other type to the other nestling. If only one nestling hatched, it was given one virus and a single nestling in another nest was given the other.

In 1950, 5 nestlings were inoculated with each virus and 30 were not inocu-

lated. In 1951, 20 nestlings were inoculated with each virus and 30 were not inoculated. In 1952, none of the 29 nestlings was inoculated.

Samples of mites were collected periodically from each cage housing pairs of doves and their progeny, and were tested for virus by the technique utilized for field samples of mites. Samples were never collected from young at a time when artificially induced viremia would be expected, but rather at times when positive findings would be significant as an indication of successful infection and maintenance of infection in the mites.

Immature doves were bled when 30 days of age and adult birds were bled periodically, and their blood serum was tested for neutralizing antibodies to W.E.E. and S.L.E. viruses. The neutralization index was interpreted as described for chickens.

Chicken colony. After numerous unsuccessful attempts to establish colonies of *B. sylviarum* on chickens, a colony was established on baby chicks using the technique of Chamberlain and Sikes (1950). This colony was used in 1951 and 1952 to test the vector capacity of the mite for W.E.E. virus. The mite colonies were kept in an indoor insectary with temperatures held constantly above 75°F.

Virus was introduced by releasing into the mite colony chicks 5 to 14 days of age, 15 to 24 hours after their inoculation with 0.1 or 0.2 ml. of 100 LD_{50} (mouse titration) of W.E.E. virus (strain BFS 1034). After this "infecting" phase, normal baby chicks 5 to 8 days old were introduced into the mite cage. After 1 to 2 days' exposure these survivors were either bled and tested for viremia, or were removed, placed in clean cages and bled 2 weeks later and their sera tested for neutralizing antibodies.

Subsequently a series of six inoculated chicks was introduced into the same mite colony. After 24 to 48 hours' exposure, the chicks were removed and a blood sample was taken for virus tests. Immediately after the infecting phase, a series of 23 normal uninoculated chicks (3 to 11 days old) was introduced. Each was exposed to mite feeding for 1 to 6 days and then removed to a mite-free cage and held for two weeks longer. As before, after a two week period the chicks were bled and their sera tested for W.E.E. neutralizing antibodies.

At intervals during the two experiments, samples of 100 to 300 engorged mites were collected from the colony, were frozen and later were tested for virus by the procedure used in the dove experiments.

RESULTS AND COMMENT

Evidence of natural infection. Thirteen isolations of encephalitis viruses have been made in tests of over 130,000 mites from 1946 through 1949. Data pertinent to these isolations and the distribution of sampling from various bird host species are summarized in Tables 1 and 2. Four bird species were the source of infected mites: yellow-headed blackbird (*Xanthocephalus xanthocephalus*), Brewer blackbird (*Euphagus cyanocephalus*), tricolored blackbird (*Agelaius tricolor*) and English sparrow (*Passer d. domesticus*). Both W.E.E. and S.L.E. viruses were isolated, in different years, with one exception occurring in 1946; this virus, described previously (Reeves *et al.*, 1947; Hammon *et al.*, 1948), is now known to be a mixture of the two.

TABLE 1

History of virus-positive pools

Year	Bird Host Species	Mite	Number			Virus Type	Date Collected
			Mites each pos. pool	Pools virus pos.	Nests virus pos.		
1946	Yellow-headed blackbird	*B. sylviarum*	250	4	1	3 W.E.E., 1 W.E.E. & S.L.E.	6-21-46
	English sparrow	*B. sylviarum* & *D. americanus*	200	1	1	W.E.E.	6-28-46
1947	English sparrow	*B. sylviarum*	200	3	1	W.E.E.	6-3-47
	Brewer blackbird	*B. sylviarum*	250	1	1	W.E.E.	6-6-47
1948	Tricolored blackbird	*B. sylviarum*	250	3	1	S.L.E.	7-20-48
1949	Tricolored blackbird	*B. sylviarum*	100	1	1	W.E.E.	6-29-49

In three instances several isolations were made from mite samples from a single nest (Table 1): the four isolations from yellow-headed blackbird mites in 1946 represented four pools of 250 mites each collected from a single nest; the same is true of the three isolations from English sparrow mites in 1947, and the three isolations from tricolored blackbird parasites in 1948. Thus, 10 of the 13 isolations from mites represented samples from only three bird nests. The virus-positive mites were from only 6 of 223 nests sampled over a 4-year period.

In all but one instance the virus-positive pools contained only *B. sylviarum*. Knowledge of the approximate number of mites of the two species tested (*B. sylviarum* and *Dermanyssus americanus*) can be obtained in the taxonomic identification of samples taken each year from all mite-infested nests under study, including those contributing virus test pools (Table 3). The proportions of these two mite species may have varied materially in virus test pools from year to year, with the proportion of *B. sylviarum* varying from 53.3 per cent in 1948 to 96.5 per cent in 1947. If one applies the species distribution percentage for the total taxonomic sample for this 3-year period to the total of mites tested for virus in these years, approximately 85,400 *B. sylviarum* and 30,600 *D. americanus* were tested for virus.

Detailed data on the distribution of the several mite species on different hosts, seasonal variations in populations, and other phases of their biology will be published separately (Doetschman, unpublished data).

Of immediate concern to epidemiologists is the relationship in time and area between natural infections of mosquito vectors and mites. In 1946, sampling

TABLE 2

Number of mites tested for virus from nests of different bird species

Host	Year and Number Sampled									
	1946		1947		1948		1949		Total	
	Nests	Mites	Nests	Mites	Nests	Mites	Nests	Mites	Nests	Mites
English sparrow	16	9,114	22	15,438	56	30,997	15	6,495	109	62,044
House finch	3	648	19	9,601	5	3,570	1	1,000	28	14,819
Tricolored blackbird	2	2,000	3	3,105	7	4,960	14	11,124	26	21,189
Yellow-headed blackbird	3	2,400	—	—	8	6,656	9	6,381	20	15,437
Brewer blackbird	—	—	6	4,545	5	1,991	10	5,020	21	11,556
Red-winged blackbird	—	—	5	3,907	2	2,124	—	—	7	6,031
Cliff swallow	3	156	—	—	2	331	—	—	5	487
Mourning dove	—	—	1	160	1	250	—	—	2	410
Kingbird	—	—	—	—	1	925	—	—	1	925
Lark sparrow	1	1,000	—	—	—	—	—	—	1	1,000
Mockingbird	—	—	1	378	—	—	—	—	1	378
Bullock oriole	—	—	2	758	—	—	—	—	2	758
Oregon junco	—	—	1	300	—	—	—	—	1	300
Total	28	15,318	60	38,192	87	51,804	49	30,020	224	135,334

TABLE 3

Taxonomic determination of approximate number of the two kinds of mites tested for virus (831 nests examined)

Mites	Total			1947			1948			1949		
	Nests sampled	Identified mites		Nests sampled	Identified mites		Nests sampled	Identified mites		Nests sampled	Identified mites	
		Number	% total		Number	% total		Number	% total		Number	% total
B. sylviarum	340	15,488	73.6	117	6,488	96.5	113	5,130	53.3	110	3,910	82.6
D. americanus	145	5,553	26.4	12	237	3.5	85	4,494	46.7	48	822	17.4
Total		21,041			6,725			9,624			4,732	

periods for the two did not overlap sufficiently to allow interpretation. In subsequent years the first isolations from mosquitoes and mites coincided in collections made in the same ten days, or, as in 1947, the first mosquito isolations preceded those in mites by a few additional days. This coincidence is believed to have no great significance because these were years of relatively low virus activity in the arthropod vectors and vertebrate hosts, and infection was apparently quite limited in the areas in which it could be detected. In 1948, the only isolations from mites and mosquitoes were all made from the same collecting locality within a 2-day interval, yet the three isolations of viruses from mites of one bird nest were S.L.E. and the one virus isolation from a collection of *Culex tarsalis* was W.E.E.

TABLE 4

Seasonal distribution of mite samples tested for virus and virus isolations, 1946–1949

Date Collected	Number			Virus Isolations	
	Mites	Pools	Nests	W.E.E.	S.L.E.
Apr. 20–30	101	1	1		
May 1–10	2,827	15	5		
11–20	6,520	28	13		
21–31	6,687	34	18		
June 1–10	17,941	79	30	4	
11–20	15,322	75	25		
21–30	35,783	142	52	6*	1*
July 1–10	19,930	85	33		
11–20	15,521	65	25		3
21–31	7,694	38	16		
Aug. 1–10	4,220	16	7		
11–20	1,519	7	4		
21–31	1,269	11	7		
Totals	135,334	596	236	10	4

* One isolation contained both W.E.E. and S.L.E. viruses.

The samples of mites were sufficiently large and extended over a sufficient period of late spring and early summer, beginning in May, to provide opportunity to detect virus surviving through the winter in the early samples of mites if such had actually occurred (Table 4). However, the earliest isolations were made during the first ten days of June in 1947; two isolations from mosquitoes preceded this.

Further field evidence relevant to the significance of mites as vectors of the encephalitis viruses is the proportion of species of wild and domestic birds immune after natural infection. While detailed data will be published separately (Reeves, Hammon and McClure, 1953), a preliminary summary of the incidence of neutralizing antibodies in certain species of birds studied with regard to mite ectoparasites is pertinent (Table 5). It may be significant that the proportion of antibody-positive birds among the species from which virus-positive mites were collected did not differ markedly from the proportion among those from which large samples of mites were negative, such as the house finch and red-winged blackbird, or the domestic chicken on which *Bdellonyssus* or *Dermanyssus* mites could not be found even though a large number of birds and nest boxes were carefully examined. Instead, the distribution of antibodies seems to reflect the activities of a vector of catholic host selection and free movement from host to host, such as a mosquito.

Laboratory transmissions

Dermanyssus gallinae. Results of tests of the ability of this mite to become infected with and to transmit S.L.E. or W.E.E. viruses were uniformly negative, offering no evidence that this mite is important as a vector or reservoir of these viruses.

TABLE 5

Summary of the incidence of neutralizing antibodies in selected birds of Kern County, California, 1946—1949

Bird	Western Equine			St. Louis		
	Tested	Positive		Tested	Positive	
		Number	Per cent		Number	Per cent
English sparrow	433	43	10	390	19	5
House finch	149	36	24	131	21	16
Brewer blackbird	56	6	11	55	2	4
Tricolored blackbird	15	1	7	15	0	0
Red-winged blackbird	28	10	36	25	4	16
Chicken	304	40	13	111	30	27

St. Louis virus. In three experiments 4 virus-inoculated chickens were introduced into uninfected, starved colonies of mites and were left there for 48 to 72 hours. Thousands of mites fed. Each of these chickens had a viremia in one or more blood samples taken during or immediately after exposure to mite feeding.

Ten samples totaling 7,808 mites were tested at intervals between 1 and 132 days after feeding on infected chickens and these were all negative on virus tests.

In the last of the three experiments, 3 normal chickens were introduced into the colony and were fed on by thousands of mites in a 24-hour period. Twenty-four and forty-eight hours later blood samples were collected and were negative in virus tests.

Western equine virus. In two experiments 6 inoculated chickens were introduced singly or in pairs into uninfected starved colonies of mites and were left there for 18 to 72 hours. Thousands of mites fed. Five of the 6 chickens were shown to have a viremia in one or more blood samples taken during or immediately after exposure to mite feeding. Nine samples totaling 16,887 mites were tested at intervals between 2 and 30 days after feeding on infected chickens and these were all negative on virus tests.

In each experiment normal chickens (3 in Experiment 1, and 4 in Experiment 2) were introduced into the colonies and thousands of mites fed. In Experiment 1, viremia tests of 24- and 48-hour blood samples were negative. In Experiment 2, blood taken after 20 days was tested for neutralizing antibodies to W.E.E. and S.L.E. virus, with negative findings.

Bdellonyssus sylviarum. The procedures for testing the ability of *B. sylviarum* to become infected with and to transmit W.E.E. and S.L.E. viruses were sufficiently different from those for *D. gallinae* so that the results will be presented by host rather than by virus.

Doves. In the first year, 1949, 14 nestlings were inoculated with W.E.E. virus and 9 with S.L.E. virus. In serological tests for homologous neutralizing antibodies, 7 of the 14 birds inoculated with W.E.E. virus were tested; the results in 5 were positive and in 2 equivocal. Seven of the 9 birds inoculated with S.L.E. virus were tested; the results in 5 were positive, in 1 equivocal and in 1 negative.

Samples totaling 477 mites were collected during the summer when birds were being inoculated; all were negative in virus tests. Mites were observed on almost all young birds during these experiments and on several occasions were so numerous that young nestlings were killed by the infestation before they reached an age suitable for inoculation.

In a test for evidence of transmission by mites, blood samples taken two or more weeks after inoculation of virus were tested for the heterologous antibody, that is, the virus other than the one with which they had been inoculated. Of 12 W.E.E.-inoculated birds, none had neutralizing antibodies to S.L.E. virus. Of 9 S.L.E.-inoculated birds, 7 had no neutralizing antibodies to W.E.E. and 2 had equivocal indices (less than 50).

Uninoculated young doves were not held as controls for serological study during this summer period. Two uninoculated doves died when 6 and 15 days of age. These birds were autopsied and their brains tested for virus, with negative findings.

Two birds inoculated with virus when 7 days old died 5 days later. The first of these had been inoculated with W.E.E. virus, and a virus agent could not be isolated from its brain. The second bird had been inoculated with S.L.E. virus according to records, and a strain of W.E.E. virus was isolated from its brain. It cannot be said with any certainty that this isolation represented transmission of the virus. A blood sample was not available to test for S.L.E. antibodies to confirm that S.L.E. virus had actually been the virus inoculated. An error in inoculation records is a possibility. Of the other 4 birds inoculated with S.L.E. virus on this day, serological tests showed that in the 3 which had been bled the inoculation records were accurate. According to records the sibling of the bird from which W.E.E. virus was isolated was inoculated with W.E.E. virus on the same day; this was not confirmed by serological tests as the bird died.

There was no conclusive evidence of successful infection of mites with virus, or of transmission.

The study of transmission of the 2 viruses in the mite-infested dove colony was renewed in the early summer of 1950 and maintained through the summer of 1952. The data from this study are summarized in Table 6. Eleven pairs of doves and 136 of their progeny were included in this study. Twenty-five nestlings were inoculated with W.E.E. virus and a similar number with S.L.E. virus. Serum neutralization tests for both viruses were made on blood samples taken from all the birds except the few which had died during the experiment. Of 20 W.E.E.-inoculated birds tested, 17 had significant titers to W.E.E. and none to S.L.E. Of 21 S.L.E.-inoculated birds, 7 had positive, 12 equivocal, and 2 negative titers to S.L.E. Twenty-two S.L.E.-inoculated birds were tested for W.E.E. antibodies; 1 was positive, 1 was equivocal and 20 were negative.

There appears to be little question that almost all of the W.E.E.-inoculated birds were successfully infected, and there was no evidence of S.L.E. infection in this group. In the case of the S.L.E.-inoculated birds, some consideration must be given to interpretation of the neutralization index. Experience in this laboratory indicates that it is usually low in avian hosts experimentally infected with the strains of S.L.E. virus used in these studies. It is not unlikely that the

TABLE 6

Summary of virus transmission studies with 11 families of doves and their mite ectoparasites, 1950–1952

Family Number	Parent Numbers	Parent Antibody History		WEE Inoc. Progeny							SLE Inoc. Progeny							Uninoc. Progeny							Mites Tested
				No. inoc.	Neutralization tests						No. inoc.	Neutralization tests						No. tested	Neutralization tests						
					WEE			SLE				WEE			SLE				WEE			SLE			
		WEE	SLE		+	±	0	+	±	0		+	±	0	+	±	0		+	±	0	+	±	0	
1	44–422567	+	0																						
	44–422568	0	0	3	0	0	3	0	0	3	2	0	0	2	1	1	0	4	0	0	4	0	0	3	525
2	42–401929	+	0																						
	42–401930	0	0	3	3	0	0	0	0	3	3	0	0	3	0	3	0	19	0	1	18	0	0	19	1810
2A	D 408	0	0																						
	D 419	±	0	0							0							2	0	1	1	0	0	2	250
2B	D 420	0	0																						
	D 411	±	0	1	1	0	0	0	0	1	1							6	0	0	6	0	0	6	750
3	44–404753	0	0																						
	44–404732	0	0	2	2	0	0	0	0	2	2	0	0	2	0	1	1	5	0	0	5	0	0	5	470
4	44–404747	±	0																						
		0	0	5	3	0	0	0	0	3	5	0	1	4	2	2	1	11	0	3	8	0	0	11	515
5	44–404722	±	0																						
		0	0	6	4	0	0	0	0	4	6	0	0	6	2	3	0	17	0	0	17	0	0	15	466
6	42–401928	0	0																						
	42–401914	0	0	4	4	0	0	0	0	4	4	1	0	2	1	2	0	15	0	1	14	0	0	15	875
7	44–404800	0	0																						
	44–404749	±	0	0							0							6	0	0	6	0	0	6	119
8	D 428	0	0																						
	44–404800	0	0	0							1	0	0	1	1	0	0	1	0	1	0	0	0	1	800
9	D 421	0	0																						
	44–422583	0	±	1							1							0							1000
Total				25	17	0	3	0	0	20	25	1	1	20	7	12	2	86	0	7	79	0	0	83	7580

12 S.L.E.-inoculated birds with equivocal titers (neutralization index less than 100) had actually been infected, as 8 of the 12 had neutralization indices of over 50.

The findings of 2 birds in the S.L.E.-inoculated group with protective antibodies to the W.E.E. virus must be considered in some detail because of its possible significance in the transmission of the virus. One bird had a high W.E.E. neutralization index, greater than 2250; there is little question that this bird was infected with W.E.E. virus. Records indicate it was inoculated with S.L.E. virus, but its neutralization index to this virus was only 18. Its sibling nest mate was inoculated with W.E.E. virus on the same day and developed a W.E.E. neutralization index of 780, remaining negative to S.L.E. This one positive W.E.E. finding may represent mite transmission or, as a secondary possibility, infection from intimate contact and soiling with the freshly inoculated virus from its nest mate which could have seeped from the site of subcutaneous inoculation. Other means of contact transmission during the active phase of W.E.E. infection cannot be entirely ruled out, though they appear unlikely. Mechanical transmission by mites feeding alternately on the W.E.E.-inoculated and uninoculated birds is even possible.

The S.L.E.-inoculated bird with an equivocal index (25) to W.E.E., developed no antibodies to S.L.E. Its sibling was inoculated on the same day with W.E.E. virus and developed a neutralization index of 1180, remaining negative to S.L.E.

Of the 86 uninoculated young doves in the colony, 83 were tested for neutralizing antibodies to S.L.E.; all were negative. All 86 were tested for W.E.E. antibodies, the results for 78 were negative and 7 were equivocal. The individual equivocal neutralization indices were 14, 27, 32, 34, 37, 54 and 98. Only the last one of these indices would be highly suspected of having resulted from W.E.E. infection. Artificial infections had not been carried out in the colony for the five months preceding these bleedings. The other equivocal indices are of very questionable significance because they are usually higher in infected birds. The equivocal indices did not result from transovarian transmission of antibodies from immune parents: only two parent birds in all the colony had significant titers of W.E.E. antibodies (as a result of infections preceding this experiment). The young bird with a titer of 98 was the progeny of serologically positive adults, but the young bird retained a serologic response when over six months of age by which time maternal antibodies would have disappeared (Reeves *et al.*, 1954).

Mite samples were collected for virus tests at frequent intervals during 1950 and 1951. The total number of mites tested over the 2-year period was 7,580, and virus was not isolated. In view of these findings the rare, apparent transmission may well have been accomplished mechanically by contaminated mouthparts of a mite interrupted in its feeding on a bird with viremia; this has been demonstrated with mosquitoes and W.E.E. virus by Barnett, 1954.

Observations were made of the presence or absence of mites on all nestlings. Mites were observed on 46 of 50 birds inoculated with W.E.E. or S.L.E. viruses and on 56 of 86 uninoculated birds. Undoubtedly, mites occurred on a larger

proportion of the latter group than is indicated because mites in very small numbers might not be observed, and fairly heavy infestations were actually those most likely to be recorded.

Chickens. The *B. sylviarum* colony established on baby chicks was tested for its ability to become infected with and to transmit W.E.E. virus. Five inoculated chicks were introduced into the colony. All died within 2 to 5 days and samples of blood were not obtained. Many engorged mites were observed. After this, 8 uninoculated chicks were introduced into the colony, each being left for 1 to 2 days. Two chicks died, 2 were negative on 48-hour viremia tests and 4 had no W.E.E. neutralizing antibodies in 15- to 17-day blood samples. A sample of 120 mites, collected 14 days after removal of the last inoculated chick, was negative on virus test.

In a second attempt to infect the mite colony, 6 inoculated chicks were introduced into the colony. Virus was isolated from the blood of 5 of the 6 chickens after 1 to 2 days' exposure to mite feeding. During the next 44 days, 23 uninoculated chicks were placed in the mite colony, each being exposed to mite feeding for 1 to 6 days. Serum neutralization tests of 17 birds bled after a 10- to 14-day holding period were negative. The other birds died before blood was taken.

Ten mite samples were collected for virus tests, the first being taken 24 hours after the last inoculated bird was removed from the colony, and subsequent samples were taken at intervals up to 46 days later. A total of 1,450 mites were tested, with no virus isolations. Three samples of 250 mites each, collected from the colony stock preceding these experiments, were also negative on virus tests.

DISCUSSION

The reviewed and presented evidence has well established the fact that bird mites, from domestic or from wild avian hosts, may harbor three types of encephalitis viruses in some endemic areas of North America. This is a primary step towards their incrimination as vectors of such viruses. It remains to be proven that these mites are biologically capable of maintaining an infection with or of transmitting these viruses.

In contrast to the findings of virus-infected mites in certain endemic areas, rather extensive field evidence from other endemic areas suggests that these mites may not be essential vectors or reservoirs of infection. In these instances tests of large samples of *D. gallinae* and of its frequent associate on domestic birds, *D. americanus*, collected from domestic fowl, revealed no virus. These studies encompass endemic areas in Washington (Hammon, Reeves *et al.*, 1942; Hammon and Reeves, 1947; Reeves, Hammon *et al.*, 1952), Minnesota (Burroughs *et al*, 1954), Oklahoma (Reeves *et al.*, 1947), and North Dakota (Eklund, 1954). Over 500 pools of mites, including hundreds of thousands of specimens, have been examined. All were taken from actively endemic areas and were tested by as sensitive isolation techniques as were used for pools proven to be positive in other areas, and effective at the same time in isolating virus from mosquitoes. In addition, in the highly endemic area of California covered by this study, *D. gallinae* has not been found associated with domestic birds.

In the case of *B. sylviarum* and *D. americanus* from wild birds, in general

only a small proportion of pools tested was found infected. Furthermore, 10 of the 13 isolations came from only 3 nests, emphasizing that few nests contain mites with virus. If transmission was congenital and this was an important source of infection, a higher incidence might be expected. The finding of several pools containing virus in a single nest on rare occasions could equally well or perhaps better be explained by recent feeding on a single infected bird in the nest. Most positive pools were collected when there was evidence of concurrent, and even preceding virus activity in the mosquito vectors. Extensive studies of these mites in two endemic areas, Washington (Reeves, Hammon *et al.*, 1952) and Minnesota (Burroughs and Burroughs, 1954), have revealed no viruses.

The years during which these studies were made were characterized by slight virus activity in vertebrate hosts and mosquito vectors. If mites were essential reservoirs or vectors of these viruses in the endemic areas studied one might expect, even during such years, to find convincing supporting evidence.

The transmission trials reported in this study add no evidence that these mites can transmit or even harbor these viruses. Rather, the findings appear similar to those in studies of Newcastle disease virus in *B. sylviarum* in which at best the virus persisted for only a few days after ingestion (Hofstad, 1949).

It must be concluded that neither the field observations alone nor the laboratory experiments furnish convincing or conclusive evidence of the importance or essentiality of these species of mites as vectors or reservoirs of W.E.E. or S.L.E. viruses in most of the endemic areas studied. There is no obvious explanation of the divergence between these findings and those with *D. gallinae* and S.L.E. virus in the vicinity of St. Louis as reported by Smith and her associates.

SUMMARY

Virus tests of over 130,000 *Bdellonyssus sylviarum* and *Dermanyssus americanus* collected from wild bird nests in Kern County, California, from 1946 through 1949, resulted in 9 isolations of Western equine encephalomyelitis virus, 3 of St. Louis encephalitis virus, and in 1 instance isolations of both viruses from a single sample pool. The infected mites, principally *B. sylviarum*, were collected from nests of four different bird species: yellow-headed blackbird, Brewer blackbird, tricolored blackbird and English sparrow. Virus isolations did not correlate with the bird species from which the largest mite samples were collected, nor with the distribution of neutralizing antibodies in six common species of birds.

Tests of the ability of *Dermanyssus gallinae* and *B. sylviarum* to become infected with and to transmit St. Louis encephalitis or Western equine encephalomyelitis viruses gave no conclusive evidence of successful long-term infection of these mites with virus or of biologic transmission.

The importance of these two mite species as reservoirs or vectors of the two encephalitis viruses studied in California and other western areas must be seriously questioned.

ADDENDUM

Unreported studies on *D. gallinae* as an experimental vector of Japanese B encephalitis virus are of possible significance in a complete consideration of the

role of this mite as a vector of encephalitis viruses. In this study the same basic techniques were employed as described in this paper. Mites were allowed to feed on three inoculated birds, two of which were shown to have virus circulating in their blood. Virus tests of 4,602 mites subsequently collected from the colony were negative.

The only extensive search for natural infection of mites with Japanese B virus has not been published (Hammon and Tigertt, 1953). In a study in endemic areas of Japan, 57,453 mites were collected from 200 bird nests during the summer months and virus could not be isolated. These mites were mostly from 188 nests of the house swallow, *Hirundo rustica*, and a few from the tree sparrow, *Passer montanus*, and the grey wagtail. A previous, more limited study of 170 *D. gallinae* from domestic birds was also negative (Sabin, 1950).

ACKNOWLEDGMENT

The authors are indebted to the following persons for their assistance: in transmission studies, R. E. Bellamy, R. W. Bunn, D. C. Thurman, E. M. French and C. España; in field studies, D. P. Furman and B. Brookman; and laboratory tests, J. S. Hollis.

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LABORATORY INVESTIGATIONS ON THE ROLE OF BIRD MITES IN THE TRANSMISSION OF EASTERN AND WESTERN EQUINE ENCEPHALITIS[1]

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That bird mites may play a role in the maintenance and transmission of the arthropod-borne encephalitides has been suggested by several workers. Smith, Blattner and Heys (1944) found the common chicken mite, *Dermanyssus gallinae* (DeGeer), naturally harboring St. Louis virus. Subsequently (1945, 1946) they showed that this species could be infected in the laboratory and could pass the virus through its eggs to following generations. They also reported transmission of the virus to chickens by the bites of infected mites and infection of normal mites from mite-infected chickens (1947).

These findings, together with field isolations of western equine encephalitis (WEE) from several species of mites [*Bdellonyssus sylviarum* (C. & F.) (Reeves et al, 1947), *D. gallinae* (Sulkin, 1945), *B. bursa* (Berlese) (Sulkin and Izumi, 1947), *D. americanus* Ewing (Miles *et al.*, 1951), and of the eastern type (EEE) from *D. gallinae* (Howitt *et al.*, 1948)], lent substance to the belief that mites may be arthropod reservoirs of these viruses, serving to keep the infections alive from season to season and furnishing them foci from which to spread each year.

The present study was conducted in an effort to determine whether three common species of bird mites, *D. gallinae*, *B. sylviarum*, and *B. bursa*, could be infected in the laboratory with EEE and WEE viruses and transmit them by bite to susceptible host animals. Their ability to carry the viruses by trans-ovarian passage was also studied.

MATERIALS AND METHODS

Mites. The strains of *D. gallinae* and *B. sylviarum* used were colonized from specimens collected in Colorado and Kansas, respectively, by Mr. Virgil I. Miles of the U. S. Public Health Service. The *B. bursa* colony originated from mites collected near Montgomery, Alabama. Methods of maintaining colonies of these species have been described previously (Chamberlain and Sikes, 1950). All incubations were at 80° to 85°F. and 60 to 70% relative humidity.

For infection of the mites, New Hampshire Red chicks were inoculated subcutaneously with virus when ½ to 2½ days old and used to feed the mites a day later when the viremia in the infected chick is usually at peak. Blood samples for titration intracerebrally in mice were taken from the chicks at the time of mite feeding. Engorged mites were transferred by aspirator to clean chambers

[1] The authors are grateful to Miss Janice Cailleteau and Miss Roberta M. Butler for technical assistance.

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and maintained as colonies during the course of an experiment. Unless otherwise indicated, freshly hatched (½ day old) New Hampshire Red chicks were used as host animals in mite transmission attempts, as these were found to be extremely susceptible to EEE and WEE virus infection (Chamberlain *et al.*, 1954).

In attempted isolations of EEE and WEE viruses from mites, each lot was ground in a mortar in 1.0 ml. of 30% normal horse serum, treated with 2.0 mg. of streptomycin and 1,000 units of penicillin, and inoculated either intracerebrally in 3-week-old CFW mice, or subcutaneously in ½-day-old New Hampshire Red chicks, or in both mice and chicks. On occasion, the 1.0 ml. mite suspensions were titrated in mice or chicks to determine mite virus levels.

Virus. The strain of EEE virus used throughout was one isolated from *Culiseta melanura* (Coq.) (Chamberlain *et al.*, 1951) and had two mouse brain passages and one chick embryo passage. The strain of WEE was also from *C. melamura* (Kissling *et al.*, 1954) and had two mouse brain passages.

EXPERIMENTAL

Eastern equine encephalitis

Dermanyssus gallinae. Experiment 1. Approximately 8,000 adult mites were fed upon two 2½-day-old chicks which were circulating EEE virus in a titer of $10^{-5.0}$. The engorged specimens were then transferred to a clean mite rearing chamber. At this time 500 female mites were segregated and their eggs collected. Protonymphs hatching from these eggs were tested for virus as evidence of transovarian passage. Eighteen samples of 100 mites each for mouse inoculation were withdrawn from the "infected" colony during the first 26 days at daily intervals or twice weekly. Ten additional lots of 1,000 mites each were tested on the 31st day, and 2 more lots of 1,000 on the 44th day. Three normal chicks were furnished the colony once each week to feed the mites and for attempted transmissions by mite bite. One chick was sacrificed at approximately 24 hours, the second at 48 hours, and the third at 72 hours and their bloods and brains were checked for presence of virus by intracerebral mouse inoculation. A total of 12 chicks was used, all of which were ½-day-old except for 3 introduced on the 13th day of mite incubation when 1½-days-old.

Virus was isolated from mite lots for the first 4 days after the infected meal and on the 15th day, but from none of the others. No virus was recovered from 2,000 protonymphs developed from eggs laid as a result of the infected blood feeding and tested on the 6th day. Mass colony feedings upon the 12 normal chicks resulted in one virus transmission. In this instance, one of the chicks, introduced into the colony on the 26th day and tested 72 hours later, had a small quantity of virus in the brain (1 mouse of 6 inoculated succumbed), but not in the blood. Passage and neutralization tests verified EEE virus infection.

Experiment 2. Approximately 10,000 adult mites were fed upon 4 one-day-old chicks with EEE blood virus titers of $10^{-5.0}$ to $10^{-5.7}$ and transferred to a clean rearing chamber. Lots of 10 mites each were withdrawn daily for the first 5 days and titrated in mice to determine the relative amounts of virus present on suc-

cessive days. Other lots of 100–300 mites were withdrawn daily during the first 10 days and then twice or once weekly over a 76-day period and tested qualitatively for presence of virus by mouse and chick inoculation. A pool of 750 protonymphs produced as a result of the infected meal was also screened for virus as evidence of a transovarian passage. Three normal chicks were introduced into the infected colony weekly as in the preceding experiment to feed the mites and attempt virus transmission. These were sacrificed and their bloods and brains tested for virus after 24, 48, or 72 hours exposure to mite feeding. A total of 27 chicks was used.

The excrement of 275 mites which accumulated for 6 days at 80°F. following the EEE virus blood meal was pooled in 1.0 ml. of diluent and inoculated into mice to determine its infectivity.

The virus titer of a 1.0 ml. suspension of 10 mites tested within 1 hour of the infected feeding was $10^{-5.7}$. Similar mite samples after 1 day's digestion of the infected blood titered $10^{-4.5}$, to $10^{-5.0}$; after 2 days, $10^{-3.0}$; after 3 days, $10^{-2.2}$; and after 4 and 5 days, less than 10^{0}.

Lots of 20–300 mites each were found by mouse and chick inoculation to contain virus after extrinsic incubation of 4, 5, 6, 7, 8, 10, and 15 days, but not at 13, 20, 25, 33, 40, 47, 54, 62, 68, or 76 days. Chicks proved to be the most sensitive medium for detection of virus. These results are shown in Table 1.

No transovarian passage of EEE virus was demonstrable, nor was a transmission of infection to chicks by mite bite accomplished. The attempt to isolate virus from mite excrement was also negative.

Experiment 3. Adult *D. gallinae* were permitted to engorge upon chicks with blood virus titers of $10^{-7.5}$ to $10^{-8.5}$. Within an hour of the feeding, either 1 or 2 of these mites was crushed on various body areas of normal ½-day-old chicks, such as the palate (2 mites), eye corners (1 mite per eye), nostrils (1 mite per nostril), membranous tissue of vent (1 mite), normal skin (1 mite) and lightly scratched skin (1 mite). Five chicks were used in each test. Concurrently, titration of 2 mites from the same lot revealed them to contain $10^{5.5}$ and $10^{6.3}$ mouse intracerebral LD_{50} of virus, respectively.

The results, summarized in Table 2, showed that infection in chicks could be caused by crushing infected mites upon various body surfaces. Only the normal-skin and eye-corner tests failed to bring about infection in at least 1 chick out of 5.

Bdellonyssus bursa. Experiment 1. Approximately 10,000 adult and 10,000 protonymph *B. bursa* were fed upon chicks with EEE virus in their blood in titers of $10^{-8.0}$ to $10^{-8.5}$ and transferred to a rearing chamber. Three normal chicks were supplied each week over a 4-week period to feed the mites and to attempt virus transmission by mite bite. The bloods and brains of these chicks were tested for virus after 1, 2, or 3 days of exposure to mite bites. Lots of 100 mites each, mostly adults, were withdrawn from the "infected" colony for mouse inoculation, daily for the first 10 days and then 2 or 3 times weekly through the 30th day. On the 35th day, 6 lots of 1,000 mites each were withdrawn and tested to conclude the experiment. Protonymphs hatched from eggs laid as a result of

TABLE 1

EEE virus in adult Dermanyssus gallinae *at various periods after feeding upon infected chicks*

No. Mites in Pool	Incubation	Mite Generations Represented	Virus Isolated (Dead/Inoculated)		Titer of 1.0 ml. Suspension of 10 Mites, in Mice*	No. Mites in Pool	Incubation	Mite Generations Represented	Virus Isolated (Dead/Inoculated)	
			Mice	Chicks					Mice	Chicks
10	1 hr	Original	+ (6/6)		5.3	100	8 days	Original	− (0/8)	− (0/3)
10	1 hr	Original	+ (6/6)		5.7	10	10 days	Original	− (0/6)	
10	1 hr	Original	+ (6/6)		5.7	20	10 days	Original	− (0/8)	− (0/3)
10	1 hr	Original	+ (6/6)		5.3	100	10 days	Original	+ (1/8)	+ (1/3)
10	1 day	Original	+ (6/6)		5.0	100	13 days	Original	− (0/8)	− (0/3)
10	1 day	Original	+ (6/6)		4.5	30	15 days	Original	− (0/8)	− (0/3)
10	2 days	Original	+ (6/6)		3.0	100	15 days	Original	+ (2/7)	+ (2/3)
50	2 days	Original	+ (10/10)	+ (3/3)		100	20 days	Original + 1	− (0/8)	− (0/3)
10	3 days	Original	+ (6/6)		2.2	130	25 days	Original + 1	− (0/8)	− (0/3)
50	3 days	Original	+ (9/9)	+ (3/3)		300	33 days	Original + 2	− (0/8)	− (0/3)
10	4 days	Original	− (0/3)		<0.0	300	40 days	Original + 3	− (0/8)	− (0/3)
100	4 days	Original	+ (4/10)	+ (3/3)		300	47 days	Original + 4	− (0/8)	− (0/3)
10	5 days	Original	− (0/8)		<0.0	300	54 days	Original + 5	− (0/10)	− (0/3)
20	5 days	Original	− (0/8)	+ (1/3)		300	62 days	Original + 6	− (0/10)	− (0/3)
100	5 days	Original	+ (10/10)	+ (3/3)		300	62 days	Original + 6	− (0/8)	− (0/3)
10	6 days	Original	− (0/10)	+ (1/3)		300	62 days	Original + 6	− (0/8)	− (0/3)
20	6 days	Original	− (0/10)	− (0/3)		300	68 days	Original + 6	− (0/10)	− (0/3)
120	6 days	Original	+ (5/6)			300	68 days	Original + 6	− (0/8)	− (0/3)
155	6 days	Original	− (0/6)			300	68 days	Original + 6	− (0/9)	− (0/3)
10	7 days	Original	− (0/7)			500	76 days	Original + 7	− (0/10)	− (0/3)
20	7 days	Original	− (0/8)	+ (1/3)		500	76 days	Original + 7	− (0/10)	− (0/3)
100	7 days	Original	− (0/8)	− (0/3)		500	76 days	Original + 7	− (0/10)	− (0/3)
10	8 days	Original	− (0/8)			500	76 days	Original + 7	− (0/7)	− (0/3)
20	8 days	Original	+ (2/8)	+ (2/3)		400	76 days	Original + 7	− (0/8)	− (0/3)

* Titers expressed as inverse log of dilution killing half the animals inoculated. In calculating the titers, the 1.0 ml mite suspensions were considered undiluted virus material, or 10^0 dilution.

TABLE 2

EEE transmission to chicks by crushed infected Dermanyssus gallinae

Area on Which Crushed	Infections/Attempts
Palate	1/5
Eye corners	0/5
Nostrils	2/5
Vent	3/5
Normal skin	0/5
Scratched skin	3/5

the adults' infected blood meal, as well as protonymphs from eggs laid after the 1st, 2nd, and 3rd normal feedings, were tested for virus as evidence of transovarian passage.

EEE virus was isolated from the pools of mites for the first 5 days, but not thereafter. No EEE virus transmissions to the normal chicks exposed in groups of 3 on the 5th, 12th, 19th, and 26th days were accomplished. A single pool of 125 protonymphs resulting from the parent mites' infected blood meal was virus negative, as were 3 pools of 1,000 protonymphs each resulting from subsequent normal blood meals.

Experiment 2. Approximately 4,000 adult *B. bursa* and 1,000 protonymphs were fed upon infected chicks with virus in their bloods in titers of $10^{-7.5}$ to $10^{-8.4}$, and then were transferred to a rearing chamber. Eight normal chicks were furnished the "infected" mite colony in groups of 4 each, the first group on the 4th day and the second on the 11th day after the infected blood meal. One chick of each group was sacrificed and blood and brain were tested for virus by mouse inoculation on the 2nd day of mite exposure, a second chick on the 3rd day, a third on the 5th or 6th day, and the fourth on 5th to 10th day, all with negative results. One lot of 100 adult mites taken from the "infected" colony on the 5th day after the infected meal, and 2 lots of 100 each on the 11th day were negative for virus when inoculated intracerebrally into mice. On the 17th day, however, when the experiment was terminated, 3 of 6 lots of 1,000 mites each (mixed stages) contained virus. A single pool of 1,500 protonymphs from eggs laid as a result of the 1st normal chick blood meal, and tested on the 11th day following the virus meal of the parent mites, failed to yield virus.

Bdellonyssus sylviarum. About 3,500 mites, three-fourths of which were adults and the remainder protonymphs, were fed upon infected chicks with EEE virus in their blood in a titer of $10^{-8.0}$ and then transferred to a rearing chamber Normal chicks in groups of 3 were placed in this colony on the 5th, 12th, 19th and 26th days to feed the mites and to attempt transmissions of virus by mite bite. The blood and brain of the first chick of each group were tested for virus after 24 hours exposure to the mites, the second, after 48 hours, and the third, after approximately 72 hours. Lots of 50 mites each were taken from the colony and tested for virus on the 1st and 2nd days after the infected meal, and lots of 100 mites thereafter at daily or twice-weekly intervals through the 26th day. On the 31st and 32nd days a total of 850 was tested and the experiment was

terminated. One group of 300 unfed protonymphs, from eggs laid as a result of the parent mites' first noninfected blood meal, were tested in an attempt to demonstrate transovarian passage of virus.

EEE virus could be isolated from the mites for the first two days only. None of the 12 chicks exposed to mite bites developed EEE virus infection. No evidence of a transovarian passage of virus was obtained.

Western equine encephalitis

Dermanyssus gallinae. Experiment 1. An "infected" colony was started with 2,000 adult mites engorged upon chicks with WEE blood virus titers of $10^{-7.5}$ to $10^{-8.0}$. A procedure similar to that for EEE virus was followed, with 3 normal chicks placed in the colony weekly to attempt transmissions by mite bite. Mites in lots of 3 to 10 each were withdrawn from the colony daily for the first 6 days and titrated by subcutaneous inoculation of chicks and intracerebral inoculation of mice to measure the rate of virus decrease. Larger lots were taken from the colony twice weekly from the 9th to the 64th day for titration. Protonymphs from eggs laid as a result of the infected meal were tested also, as well as excrement of 50 adult mites accumulated for 6 days at 80°F. following the infected meal.

One apparent transmission to a chick by mite bite occurred on the 13th day of extrinsic incubation. This chick was sacrificed after 24 hours exposure to the mites and its blood and brain suspension inoculated subcutaneously into chicks and intracerebrally into mice. None of the mice became ill, but 2 of 4 chicks inoculated with the blood died. Passage and neutralization tests verified that death resulted from WEE virus infection. None of 17 other chicks exposed in groups of 3 to the mites each week through 41 days contracted infection.

The results of testing various mite lots for virus are shown in Table 3. Large quantities of virus, corresponding to the amounts ingested, were recovered on the day of the infected feeding. The quantity of virus present decreased daily until it reached minimum levels for detection on the 5th day. No virus was isolated beyond the 5th day from adult mites except for a trace from 25 specimens on the 9th day. Neither was virus isolated from 200 unfed protonymphs tested for evidence of transovarian passage. A small amount of virus was detected in mite excrement accumulated from 50 mites for 6 days following the infected meal.

Experiment 2. Adult *D. gallinae* were fed upon a WEE-infected chick with a blood virus titer of $10^{-7.5}$. Within an hour of the infected blood meal, one of the engorged mites was titered and found to contain $10^{5.5}$ mouse LD_{50} of virus. Others in the same feeding group were crushed upon various body areas of normal ½-day-old chicks as follows: palate, 2 mites; eye corners, 1 mite per eye; nostrils, 1 mite per nostril; membranous tissue of vent, 1 mite; normal skin, 1 mite; and lightly scratched skin, 1 mite. Four to 7 chicks were used in each test.

The results are given in Table 4. Crushing of infected mites even upon normal skin caused infection in 2 out of 4 chicks. Only when mites were crushed in the nostrils did failure to cause infection occur.

TABLE 3

WEE virus in Dermanyssus gallinae *at various periods after feeding upon infected chicks*

No. Specimens in Pool	Stage*	Incubation	Titer† of 1.0 ml. Mite Suspension in Chicks	Titer of 1.0 ml. Mite Suspension in Mice	No. Specimens in Pool	Stage	Incubation	Titer of 1.0 ml. Mite Suspension in Chicks	Titer of 1.0 ml. Mite Suspension in Mice
3	A	1 hr	6.7	4.5	10	A	6 days	<0.0	
3	A	3 hrs	6.5		10	A	6 days	<0.0	
10	A	1 day	6.0		10	A	6 days	<0.0	<0.0
10	A	1 day	6.5		200	P	6 days	<0.0	<0.0
10	A	2 days	4.5		50	AE	6 days	0.7	<0.0
10	A	2 days	4.0		25	A	9 days	<0.0	<0.0
10	A	2 days	3.6	0.8	25	A	9 days	Tr‡	<0.0
10	A	3 days	3.3		50	A	12 days	<0.0	<0.0
10	A	3 days	1.8		100	D & A	17 days	<0.0	<0.0
10	A	3 days	4.2		100	D & A	20 days	<0.0	<0.0
10	A	3 days	2.8		100	A	24 days	<0.0	<0.0
10	A	4 days	4.4		100	A	27 days	<0.0	<0.0
10	A	4 days	3.3	0.7	100	A	31 days	<0.0	<0.0
10	A	4 days	2.4		100	D & A	34 days	<0.0	<0.0
10	A	4 days	3.8		100	D & A	38 days	<0.0	<0.0
10	A	4 days	2.8	Tr.‡	100	D & A	41 days	<0.0	<0.0
10	A	5 days	0.3	<0.0	3000	P, D & A	48 days	<0.0	<0.0
10	A	5 days	1.5		3000	P, D & A	49 days	<0.0	<0.0
10	A	5 days	<0.0		3000	P, D & A	50 days	<0.0	<0.0
10	A	5 days	<0.0		2500	P, D & A	53 days	<0.0	<0.0
10	A	5 days	<0.0	<0.0	2500	P, D & A	55 days	<0.0	<0.0
10	A	6 days	<0.0	<0.0	2000	P, D & A	58 days	<0.0	<0.0
10	A	6 days	<0.0		1000	P, D & A	64 days	<0.0	<0.0

* A = adult; P = protonymph; AE = adult excrement; D = deutonymph.

† All titers expressed as inverse log of dilution killing half the animals inoculated. In calculating the titers the 1.0 ml mite suspensions were considered undiluted virus material, or 10^0 dilution.

‡ Tr. = trace (only 1 of 4 animals died receiving the original mite suspension (10^0 dilution).

TABLE 4

WEE transmission to chicks by crushed infected Dermanyssus gallinae

Area on Which Crushed	Infections/Attempts
Palate	3/5
Eye corners	1/4
Nostrils	0/5
Vent	1/7
Normal skin	2/4
Scratched skin	4/4

Bdellonyssus bursa. Approximately 3,000 adult mites were fed upon WEE-infected chicks with blood virus titers of $10^{-7.5}$ to $10^{-8.0}$, then were transferred to a rearing chamber. Normal chicks were placed in the "infected" colony weekly in groups of 3, sacrificed after 24, 48, or 72 hours exposure to the mites, and tested for virus by mouse and chick inoculations of blood and brain suspensions. A total of 18 chicks was used through the 41st day of mite incubation, none of which showed evidence of virus transmission by mite bite.

Titration of pools of 10 mites each at daily intervals revealed a high concentration of virus in the mites immediately after ingestion of infected blood, then a gradual decline in amount to the 5th day, when a low level could be detected by chick inoculation but none by mouse inoculation. No virus could be detected in larger samples of mites after 9 to 58 days extrinsic incubation. Eight hundred protonymphs, hatching from eggs laid as a result of the first normal blood meal of the "infected" parent mites, failed to yield WEE virus when inoculated into chicks and mice. These results are summarized in Table 5.

Bdellonyssus sylviarum. Approximately 5,000 *B. sylviarum* adults were fed upon WEE-infected chicks with blood virus titers of $10^{-7.5}$ to $10^{-8.0}$ and placed in a clean rearing chamber. Samples of mites were withdrawn periodically and titrated for virus by chick and mouse inoculation, including protonymphs hatched from eggs laid as a result of the adults' infected blood meal. As in the other experiments, normal chicks were introduced into the colony in groups of 3 at weekly intervals from 5 through 41 days to attempt transmissions of virus by mite bite.

None of 18 normal chicks exposed to mass mite feeding became infected with WEE.

Virus in the mites gradually decreased from amounts to be expected immediately after ingestion of infected blood to minimal levels after 5 days as measured by chick inoculation. Thereafter, no virus could be isolated from 9 through 50 days.

Chick inoculation revealed small amounts of virus in 3 of 4 pools of 200 protonymphs each, hatched from eggs laid as a result of the infected blood meal and tested on the 4th day following the infected meal. The amounts present were too small to produce signs of infection in mice. On the 6th day after the infected blood meal, 200 additional protonymphs were tested for virus with negative results. These data are summarized in Table 6.

TABLE 5

WEE virus in Bdellonyssus bursa *at various periods after feeding upon infected chicks*

No. Mites in Pool	Stage*	Incubation	Titer† of 1.0 ml. Mite Suspensions in Chicks	Titer of 1.0 ml. Mite Suspensions, in Mice	No. Mites in Pool	Stage	Incubation	Titer of 1.0 ml. Mite Suspensions, in Chicks	Titer of 1.0 ml. Mite Suspensions, in Mice
3	A	1 hr	6.0	3.5	100	A	17 days	<0.0	<0.0
3	A	3 hrs	6.3		100	A	20 days	<0.0	<0.0
10	A	1 day	5.7		100	A	20 days	<0.0	<0.0
10	A	1 day	5.7		100	A	27 days	<0.0	<0.0
10	A	2 days	4.7		100	A	31 days	<0.0	<0.0
10	A	2 days	4.4		100	A	34 days	<0.0	<0.0
10	A	2 days	4.6	1.8	100	A	38 days	<0.0	<0.0
10	A	3 days	3.5		100	A	41 days	<0.0	<0.0
10	A	3 days	4.0		100	P	41 days	<0.0	<0.0
10	A	3 days	5.0		500	P & A	48 days	<0.0	<0.0
10	A	3 days	3.8		500	P & A	48 days	<0.0	<0.0
10	A	4 days	2.2	0.0†	500	P & A	48 days	<0.0	<0.0
10	A	4 days	2.8		500	P & A	48 days	<0.0	<0.0
10	A	4 days	1.5		500	P & A	48 days	<0.0	<0.0
10	A	4 days	2.3		500	P & A	48 days	<0.0	<0.0
10	A	5 days	0.7	<0.0	500	P & A	48 days	<0.0	<0.0
10	A	5 days	0.5		500	P & A	48 days	<0.0	<0.0
10	A	5 days	1.4		500	P & A	48 days	<0.0	<0.0
10	A	5 days	1.5	<0.0	500	P & A	48 days	<0.0	<0.0
50	A	9 days	<0.0	<0.0	500	P & A	48 days	<0.0	<0.0
800	P	12 days	<0.0	<0.0	500	P & A	52 days	<0.0	<0.0
50	A	12 days	<0.0	<0.0	200	P & A	58 days	<0.0	<0.0

* A = adult; P = protonymph.

† All titers expressed as inverse log of dilution killing half the animals inoculated. In calculating titers, the 1.0 ml mite suspensions were considered undiluted virus material, or 10^0 dilution.

‡ Half the mice died which received the original mite suspension (10^0 dilution).

TABLE 6

WEE virus in Bdellonyssus sylviarum *at various periods after feeding upon infected chicks*

No. Mites in Pool	Stage*	Incubation	Titer† of 1.0 ml. Mite Suspensions, in Chicks	Titer of 1.0 ml. Mite Suspensions, in Mice	No. Mites in Pool	Stage	Incubation	Titer of 1.0 ml. Mite Suspensions, in Chicks	Titer of 1.0 ml. Mite Suspensions, in Mice
3	A	1 hr	3.8	1.8	10	A	4 days	4.2	2.0
3	A	3 hrs	5.0		10	A	5 days	1.5	<0.0
10	A	1 day	5.8		10	A	5 days	2.0	
10	A	1 day	6.5		10	A	5 days	1.5	
10	A	2 days	5.5		10	A	5 days	1.5	
10	A	2 days	3.6		10	A	5 days	1.7	<0.0
10	A	2 days	3.7	1.5	100	P	6 days	<0.0	<0.0
10	A	3 days	3.2		100	P	6 days	<0.0	<0.0
10	A	3 days	1.8		50	A	9 days	<0.0	<0.0
10	A	3 days	4.5		50	A	12 days	<0.0	<0.0
10	A	3 days	2.8		100	A	17 days	<0.0	<0.0
200	P	4 days	0.3	<0.0	100	A	20 days	<0.0	<0.0
200	P	4 days	<0.0	<0.0	100	A	24 days	<0.0	<0.0
200	P	4 days	1.2	<0.0	100	A	27 days	<0.0	<0.0
200	P	4 days	0.0‡	<0.0	100	A	31 days	<0.0	<0.0
10	A	4 days	2.8	0.0‡	100	A	34 days	<0.0	<0.0
10	A	4 days	1.3		100	A	41 days	<0.0	<0.0
10	A	4 days	3.5		500	P & A	50 days	<0.0	<0.0
10	A	4 days	0.0‡		500	P & A	50 days	<0.0	<0.0

* A = adult; P = protonymph.

† All titers expressed as inverse log of dilution killing half the animals inoculated. In calculating the titers, the 1.0 ml mite suspensions were considered undiluted virus material, or 10^0 dilution.

‡ Half the animals died which received the original mite suspension (10^0 dilution).

DISCUSSION

The experiments reported above were designed to give the three mite species optimum opportunity to become infected with EEE and WEE viruses, and, by mass refeedings upon normal ½-day-old chicks, optimal conditions for transmitting infection by bite. It is notable that, with but few exceptions, virus could not be isolated from mites beyond the first few days following ingestion of infected blood. Transmission of infection to baby chicks by bite and transovarian passage of virus, while apparently possible, were rare occurrences.

On one occasion, a small amount of WEE virus was isolated from the excrement of 50 *D. gallinae* accumulated at 80°F. for the first 6 days following their infected blood meal. This finding weakens the significance of isolating small amounts of WEE virus from pools of protonymphs of *B. sylviarum* hatched from eggs laid as a result of the parent mites' infected blood meal, for it is possible that the virus of these protonymphs may have been external through contamination rather than internal through transovarian passage.

These laboratory findings corroborate previously reported isolations of EEE and WEE viruses from mites collected in nature, as they demonstrate that mites retain virus in diminishing concentrations for several days following an infected meal. An occasional specimen may retain infection for considerably longer periods, as evidenced by isolation of EEE virus from *D. gallinae* after 15 days incubation and a transmission to a chick on the 26th day, and isolation from *B. bursa* on the 17th day. One pool of *D. gallinae* yielded WEE virus on the 9th day of incubation and a transmission was accomplished by the same "infected" colony on the 13th day. Should a nestling bird, or adult bird attending a nest, become infected from a mosquito, the mites in the nest would have opportunity for infection similar to that provided experimentally. It would be surprising if collections of mites from numerous nests at a time when encephalitis was widespread did not occasionally yield virus isolations.

The finding that mites freshly engorged upon EEE- or WEE-infected chicks could cause infection when crushed upon various body parts of normal chicks reveals a possibility of spread of infection among young birds within a nest in nature. In addition, this proof of mechanical transmission casts doubt upon the veracity of the two virus transmissions by mite bite accomplished in this investigation, as it is possible that infected mites crushed or eaten by the chicks may have been the source of infection rather than the mite feeding.

These studies lead to the conclusion that even if bird mites are capable of becoming infected and of transmitting infection by bite or by transovarian passage, the infrequency with which these happenings are accomplished in the laboratory, and probably occur under natural conditions, minimize the mite's importance in cyclic perpetuation of EEE and WEE viruses in nature. A more efficient method of maintenance of these viruses certainly must exist, as strongly indicated in studies with mosquito vectors. It must be conceded, however, that other strains or species of mites and other strains of EEE and WEE viruses than those used in this investigation possibly could form more efficient virus-vector combinations.

SUMMARY

Large numbers of *Dermanyssus gallinae*, *Bdellonyssus bursa*, and *B. sylviarum* were fed upon chicks infected with the viruses of eastern and western equine encephalitis, then refed periodically in mass upon normal chicks. Samples of mites were tested for virus at different periods of incubation. Chicks ½-day-old proved to be more sensitive in detecting virus than mice.

The viruses usually could not be recovered from the mites for more than a few days following the infected blood meal. After numerous trials involving mass feedings of many thousands of mites upon 125 chicks, two apparent virus transmissions to chicks by mite bite were accomplished, one of EEE by *D. gallinae* after 26 days extrinsic incubation, and one of WEE by this same mite species after 13 days extrinsic incubation.

On one occasion, a trace of WEE virus was detected in excrement of 50 *D. gallinae*, tested after 6 days accumulation at 80°F. following an infected blood meal. A low concentration of WEE virus was also recovered from *B. sylviarum* protonymphs hatched from eggs laid as a result of an infected blood meal by parent mites. All other similar tests were negative.

In view of the low susceptibility of these three species of mites to EEE and WEE infection, it is concluded that any role they might play in perpetuating these viruses in nature must be minor.

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MITES AS POSSIBLE VECTORS OR RESERVOIRS OF EQUINE ENCEPHALOMYELITIS IN TEXAS[1, 2]

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INTRODUCTION

Soon after the discovery of the Western equine encephalomyelitis (W.E.E.) virus over two decades ago, a search was begun for reservoir hosts and vectors of the disease. The mosquito was the first of the blood-sucking insects to be incriminated. Following the discovery that the yellow fever mosquito, *Aedes aegypti*, was capable of transmitting the virus of W.E.E. from one laboratory animal to another, numerous reports of laboratory transmission involving a variety of species of mosquitoes appeared in the literature. Hammon and his associates (1941) recorded the isolation of the W.E.E. virus from naturally infected *Culex tarsalis* mosquitoes in Yakima Valley, Washington, and later from other species. Since then the isolation of this virus from naturally infected mosquitoes has been repeated over 250 times in widely separated areas in the western part of the United States and in Canada, indicating an exceedingly high incidence of infected mosquitoes during epidemic periods. Thus the case for the mosquito as an important vector in the transmission of equine encephalomyelitis has been well-documented. The virus, however, has never been isolated from over-wintering adult mosquitoes, and it has not been transferred from generation to generation by transovarian passage. A number of potential vectors have been investigated, therefore, in order to find the true reservoir host maintaining the virus during interepidemic periods. In laboratory studies, Syverton and Berry (1941) demonstrated infection of the tick, *Dermacentor andersoni*, with the virus of equine encephalomyelitis by permitting them to feed on infected animals. They also succeeded in transmitting the disease in the laboratory from infected ticks to susceptible animals. Transovarian passage of the virus in the tick could also be demonstrated. This tick, however, has never been incriminated in any epidemic of Western equine encephalomyelitis. A related tick, *Dermacentor variabilis*, which is more widely distributed in this country did not become infected after it

[1] These studies were supported by a grant from the Rose Lampert Graff Foundation, Los Angeles, California, and the Caruth Foundation, Dallas, Texas.

[2] While these studies were in progress numerous persons assisted in the collection of specimens for analysis. For their kind cooperation the authors are indebted to Mr. George L. Carpenter, Typhus Control Supervisor, U.S.P.H.S.; Drs. H. H. Casper and T. J. Anderson, State Livestock Sanitary Commission; Dr. A. F. Davis, City Veterinarian, Beaumont, Texas. For technical assistance the authors are indebted to George C. Patterson and Cleo Hausman.

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had been allowed to feed on animals inoculated with the W.E.E. virus. Further study with *Dermacentor variabilis* seems indicated because of the ease with which it could be infected with the St. Louis encephalitis (S.L.E.) virus (Blattner and Heys, 1944).

Another group of potential vectors was suggested by the isolation of the W.E.E. virus from the kissing bug, *Triatoma sanguisuga*, collected from pastures where horses had died of encephalitis the previous year (Grundmann *et al.*, 1943). Transmission of this virus from guinea pig to guinea pig by means of this triatomid was demonstrated. *Triatoma sanguisuga* does not appear to extend to the Yakima Valley region in the State of Washington and hence has not been incriminated in that endemic area. Studies with the fowl tick, *Argas persicus*, and the bed bug (*Cimex lectularius*), collected in chicken houses, would suggest that these ectoparasites are of little significance in the biological infection chain (Sulkin, 1945; Sulkin and Izumi, 1947; Hammon *et al.*, 1945a, 1945b). In studies conducted in the San Joaquin Valley of California, Hammon and his associates (1945b) were unable to demonstrate a neurotropic virus in chicken ticks (*Argas persicus*) yet *C. tarsalis* mosquitoes collected in the same chicken houses were frequently found to be infected. In one instance those workers demonstrated that the W.E.E. virus survived for 15 days in ticks which were allowed to feed on experimentally inoculated chickens. Since experimental transmission of the disease could not be effected, their role as vectors was discounted. It is of interest, too, that no infected chicken ticks could be demonstrated by us (Sulkin, 1945) in chicken houses from which mites (*Dermanyssus gallinae*) infected with the W.E.E. virus had been collected. A wide variety of other arthropods and parasites have been investigated including blood-sucking Diptera and a number of bird helminths. In each instance the epidemiological implications have been equivocal or entirely negative. No studies have been reported which would indicate that endoparasites of avian hosts are capable of maintaining virus.

Accumulating knowledge has consistently shown that avian species, both domestic and wild, play an important role in the epidemiology of the arthropod-borne virus encephalitides. At the time of the first epidemic of St. Louis encephalitis, chickens were suspected of playing a part in dissemination of the infection but attempts by us (Sulkin *et al.*, 1940) to infect adult chickens or newly hatched chicks with human brain material or with mouse passage virus were largely unsuccessful. Unfortunately no attempt was made at the time to determine presence or persistence of virus in the blood stream. At about the same time, Pearson (1940) demonstrated persistence of the S.L.E. virus in the brain of newly hatched chicks for 6 days and sometimes as long as 20 days after subdural inoculation. Of greater epidemiological significance is the fact that Hammon and his associates (1946) later produced viremia in chickens and showed that the virus persisted in the blood for about 5 days. This virus, however, has never been recovered from naturally infected domestic fowls or any other avian species. The Western and Eastern equine encephalomyelitis (E.E.E.) viruses, on the other hand, have been recovered from avian species. Cox, Jellison and Hughes (1941) isolated the W.E.E. virus from the brain and spleen of a prairie chicken, *Tympanuchus cupido*

americanus shot near Rugby, North Dakota. More recently Sooter and his colleagues (1951) reported the isolation of the same virus from the circulating blood of 2 nestling red-winged blackbirds (*Agelaius phoeniceus*) and a nestling magpie (*Pica p. hudsonia*) from Weld County, Colorado. At about the same time Kissling and his associates (1951) reported the isolation of the E.E.E. virus from the blood of an apparently well, male purple grackle, *Quiscalus quiscula* shot in flight in the Manhac swamp area near Ponchatoula, La. This virus has been recovered previously on several occasions from the brains of naturally infected birds. Evidence of exposure to the equine encephalomyelitis viruses through the demonstration of neutralizing antibodies in the sera of wild and domestic birds has been reported on many occasions. Numerous avian species both wild and domestic, then, seem to play an important role in the biological infection cycle. It, therefore, seemed likely that some blood-sucking vector which does not necessarily bite man may transmit the disease to birds. The present report will summarize the results of field and laboratory studies designed to determine the role of mites as possible vectors or reservoirs of Western equine encephalomyelitis.

MATERIALS AND METHODS

Mites. When these experiments were initiated it was necessary to improvise methods for the collection, storage, laboratory maintenance, and particularly the feeding of mites under controlled conditions. Details of technique have been described elsewhere (Wisseman and Sulkin, 1947). The strains of *D. gallinae* used in the transmission experiments were colonized from specimens collected within the Dallas city limits. Collection and transfer of the mites was accomplished by means of a suction device with an arrangement of tubes which confined the mites to small containers which were easily detached, closed and stored. For most purposes very fine meshed bolting silk was used to close the bottom end of the storage tube since it retained all stages of the mite. In the laboratory, vacuum was obtained from an ordinary filter pump type of aspirator. In the field the same equipment was used except that suction was applied by mouth. Mites for immediate use and observation were kept in the storage vials in a chamber at room temperature and a relative humidity of approximately 70 %. When stored in the refrigerator at 4–5°C. for longer periods of time, the small storage tubes were sealed in rubber-stoppered 1 x 8 inch test tubes to prevent excessive action of moisture on the mites. It was possible to keep mites throughout the year in this manner, especially if they were fed at intervals of three to four weeks followed by a few days at room temperature to allow oviposition and hatching of ova.

The technique used for the collection of mites during the surveys was essentially the same as that described in the accompanying paper by Reeves and his associates (1955). Representative samples of mites were preserved in 70 % alcohol for toxonomic purposes.*

Feeding of mites was routinely performed on young chickens placed in Fernbach flasks covered with bolting silk. A small raised platform inside the flask

* The authors are grateful to Dr. E. P. Cheatum, Department of Biology, Southern Methodist University, for confirming some of the mite identifications.

aided in preventing the chicken from crushing the fragile engorged mites. The air current from a small electric fan prevented condensation of moisture which caused the death of many mites.

For maintaining the chicken mite colony a small coop equipped with a screened run and roosting box with hinged roof was used. Numerous wooden slats were lightly nailed about the inside of the box. Chicken mites introduced into the roosting box multiplied rapidly and could be found easily by lifting the slats with a knife-blade. This colony provided a constant source of mites throughout the summer and fall. A similar coop built indoors allowed propagation during the winter months.

Because of the difficulties encountered in attempts to colonize *L. bursa* in this laboratory no transmission studies with this species of mite were undertaken. Such studies, however, are reported in an accompanying paper by Chamberlain and Sikes (1955) who were more successful in rearing these arachnids in the laboratory. No attempt has been made by us to colonize *B. sylviarum*. Extensive studies with these mites are reported by Reeves and his coworkers (1955).

Virus testing. Details of technique used in the initial experiments for testing of mites for virus have been described elsewhere (Sulkin, 1945; Sulkin and Izumi, 1947). In studies conducted in 1948 and thereafter the supernatant fluid removed after centrifugation of triturated mite suspensions was treated with a penicillin-streptomycin mixture to rid the specimen of bacterial contaminants.

Viruses. Four strains of Western equine virus were used in these experiments. The "Olitsky" and "Howitt" strains, both obtained from Dr. W. McD. Hammon in 1943, have been maintained in this laboratory by serial mouse-brain passage; the Bennett mite virus, isolated by us from chicken mites in 1944, was available in its second and third mouse-brain passages; the Ridgeway strain, available in its second mouse-brain passage, was recovered from a fatal human case of encephalitis (Sulkin, 1946).

Neutralization tests. The technique used in the serum neutralization tests in the initial phase of the study was similar to that used by Hammon and his associates (1942) and later modified to permit more convenient assay of large numbers of specimens. The "Olitsky" strain of W.E.E. virus was used in these tests. In most instances the serums were tested after they had been in the deep-freeze storage cabinet for several months and whenever an adequate supply was available those showing questionable reactions were retested.

PREVIOUS INVESTIGATIONS

Soon after Smith and her associates (1944) reported the isolation of the S.L.E. virus from naturally infected chicken mites, *Dermanyssus gallinae*, collected in the St. Louis area, we (Sulkin, 1945) reported the isolation of the W.E.E. virus from naturally infected chicken mites collected in Dallas, Texas area at a time when an epizootic affecting a large number of horses was in progress (Sulkin, 1946). This was followed by the isolation of the E.E.E. virus from the same mite collected in Shelbyville, Tennessee at a time when sporadic cases of encephalitis in children had been reported from several areas in central Tennessee (Howitt

and others, 1948). Numerous attempts by other investigators to demonstrate neurotropic viruses in chicken mites have been negative (Reeves *et al.*, 1947, 1952; Sabin, 1950; Wenner *et al.*, 1951; Burroughs and Burroughs, 1954). No virus could be recovered from several thousand chicken mites collected by Reeves and his associates (Reeves *et al.*, 1952) over a period of many years from epidemic areas outside of California. *C. tarsalis* mosquitoes collected from many of the same chicken houses, however, were found to be infected. In addition, despite the widespread distribution of the chicken mite throughout most of the United States, this arachnid does not seem to extend to the San Joaquin Valley of California and hence has not been incriminated in an area in which annual surveys have been made. This would suggest that *D. gallinae* may not be an essential vector or reservoir in endemic areas in the Far West and further emphasizes the possible differences between the epidemiology of these infections in different geographic areas. In the search for a reservoir among other avian blood-sucking ectoparasites a number of other species of mites have been found naturally infected with neurotropic viruses (Sulkin and Izumi, 1947; Reeves *et al.*, 1947; Miles *et al.*, 1951). Since the first demonstration by us of the W.E.E. virus in chicken mites, at least 3 additional species of mites *Liponyssus bursa*, *Liponyssus sylviarum*, and *Dermanyssus americanus* have been found to be naturally infected with this virus. Table 1 summarizes, in chronological order, isolation of arthropod-borne encephalitis viruses from various species of mites found to be naturally infected.

FIELD SURVEYS IN TEXAS (1944–1948)

In the late summer and fall of 1944, with the cooperation of two local veterinarians, a survey was made of farms and ranches in the vicinity of known cases of equine encephalomyelitis in Dallas County. Between September 1 and December 1 of that year approximately 800 of 588,000 horses then in the State of Texas had encephalomyelitis. The case fatality rate was 21.7 per cent.* Three human cases were studied in our laboratory and virus was isolated from one fatal case (Sulkin, 1946). Chicken mites, fowl ticks, chicken lice and sera from chickens more and less than one year of age were collected from nine of the farms or ranches. The arthropods were kept in test-tubes for 2 to 9 days without feeding, and sera removed from the blood clots under aseptic precautions were stored in the deep-freeze storage cabinet. At the time of testing, 36 pools of arthropods were formed: 21 pools of chicken mites with 100 to 300 adults and protonymphs in each pool; 9 pools of chicken lice with several hundred in each; and 6 pools of fowl ticks with 40 to 100 in each. Using a procedure described previously (Sulkin, 1945) these collections resulted in the isolation of the W.E.E. virus from two separate pools of chicken mites collected at a ranch (Bennett ranch) north of Dallas and maintained in the laboratory for 2 and 9 days, respectively. A virus, later identified as the W.E.E. virus, was demonstrated in another pool of chicken mites collected from a nearby area. At no time was it possible to recover a virus from the fowl ticks or chicken lice.

* This information was supplied by Dr. E. A. Crist, Chief Veterinarian, Livestock Sanitary Commission of Texas.

TABLE 1

Chronological order of isolation of arthropod-borne encephalitis viruses from naturally infected mites

Virus	Mites		Location	Year	Authors
	Source	Identification			
SLE	Chickens	*Dermanyssus gallinae* (De Geer)	St. Louis, Mo.	1944	Smith, Blattner, Heys, 1944
	Chickens	*Dermanyssus gallinae* (De Geer)	St. Louis, Mo.	1945	Smith, Blattner, Heys, 1945
	Yellow-headed blackbird	*Liponyssus sylviarum* (Canestrini and Fanzago)	Kern Co., Calif.	1946	Hammon and others, 1948
	Tricolor red-winged blackbird	*Liponyssus sylviarum* (Canestrini and Fanzago)	Kern Co., Calif.	1948	Reeves and others, 1955
WEE	Chickens	*Dermanyssus gallinae* (De Geer)	Dallas Co., Texas	1944	Sulkin, 1945
	Chickens	*Dermanyssus gallinae* (De Geer)*	Tarrant Co., Texas	1946	Sulkin, unpublished observations
	Yellow-headed tricolor and Brewer's blackbird and English sparrows	*Liponyssus sylviarum*† (Canestrini and Fanzago)	Kern Co., Calif.	1946, 1947, 1949	Reeves and others, 1947, 1955
	English sparrows	*Liponyssus bursa* (Berlese)	Dallas Co., Texas	1947	Sulkin and Izumi, 1947
	English sparrows	*Dermanyssus americanus* Ewing	Weld Co., Colo.	1950	Miles and others, 1951
EEE	Chickens	*Dermanyssus gallinae* (De Geer)	Shelbyville, Tenn.	1947	Howitt and others, 1948
SLE + WEE	Yellow-headed black birds	*Liponyssus sylviarum* (Canestrini and Fanzago)	Kern Co., Calif.	1946	Hammon and others, 1948

* A filtrable agent recovered from these mites remained unidentified.

† *Dermanyssus americanus* Ewing also present in one pool of these infected mites.

In contrast to the preceding year, 1945 was a non-epidemic year for equine encephalitis in Texas. The survey conducted during this year was extended to include certain portions of Southwest Texas in addition to the Dallas-Fort Worth area. Over 50 farms and ranches were visited in six counties including Zapata, Webb, Atascosa, Kerr, Tarrant and Dallas Counties. In two areas, Zapata and Webb Counties, only fowl ticks were collected; no chicken mites could be found after a thorough search on several farms. Large numbers of chicken mites were collected on a farm at Buena Vista, Atascosa County, where a case of Western equine encephalomyelitis in a horse had occurred the previous year. No fowl ticks were observed on this farm. Blood samples from old and young (less than one year of age) chickens were collected. Only chicken mites and a few serum samples were collected within the city limits of Kerrville (Kerr County). Chicken mites, fowl ticks, and serum samples from young and old chickens were collected from several areas in Tarrant and Dallas Counties. The ticks and mites were placed in vacuum flasks with solid CO_2 and were transported to the laboratory in a frozen state. Upon arrival in the laboratory they were transferred to the deep-freeze cabinet until it was possible to test them for the presence of virus. The serum samples were also stored in the CO_2 cabinet. During the latter part of October and early part of November, 1945, a number of additional specimens of chicken mites and fowl ticks collected in various parts of the State were received for analysis. A total of 16 pools of chicken mites representing well over 6,000 specimens and 7 pools of fowl ticks (approximately 500) were tested. The results were uniformly negative. It is of special interest to note the failure to isolate virus from chicken mites collected from the identical locality where two positive isolations were made during the previous epidemic year.

Another survey was made during the summer and fall of 1946 in a four-county area surrounding and including Dallas County, when relatively few cases of the equine disease occurred in the vicinity. Thirteen pools of chicken mites (over 3,500 adults and nymphal forms), 6 pools of fowl ticks (approximately 300), and 45 serum samples from young and old chickens were collected. From each of two pools of chicken mites collected from a single area in Tarrant County a filtrable agent was recovered but remained unidentified. Inadvertently, a rack containing infected mouse brain tissue was discarded.

The failure to recover a virus from chicken mites (or fowl ticks) collected during the 1945 and 1946 surveys prompted a search for a reservoir among other avian blood-sucking ectoparasites. At a time when we were attempting to demonstrate a neurotropic virus in ectoparasites which feed on migratory birds, Reeves and his associates (1947) reported the isolation of the W.E.E. virus from wild bird mites, *Liponyssus sylviarum*, removed from the nest of a yellow-headed blackbird (*Xanthocephalus xanthocephalus*) found in Kern County, California. Later, they (Hammon *et al.*, 1948) reported on the isolation of a strain of virus with the characteristics of both W.E.E. and S.L.E. virus from mites in the same nest. While their studies were in progress we (Sulkin and Izumi, 1947) recovered the W.E.E. virus from a similar parasite, the tropical bird mite, *Liponyssus bursa*, taken from each of two nests containing fledgling English sparrows col-

lected on July 9, 1947 in the Dallas County area. In one instance the mites had been stored in a deep-freeze storage cabinet for about a month before testing. All attempts to isolate a filtrable agent from 11 additional pools of specimens of wild bird mites (representing approximately 2,500 adults and protonymphs) collected on the same day and tested within the next two weeks were negative. Twenty-one additional pools of wild bird mites (approximately 3,000) were formed from collections made the following week from 9 infested English sparrow nests. Tests conducted after these specimens had been in storage for almost two months resulted in 3 additional isolations of the W.E.E. virus. Tests for virus in 9 pools of chicken mites (approximately 2,000) and 5 pools of fowl ticks (approximately 300) collected at the same time were negative. It is of interest that none of the chicken mites found in the area where infected wild bird mites had been collected were found to be infected. Yet serum specimens from chickens in the same area contained neutralizing antibodies. In addition to 16 serum specimens from chickens collected at this time, blood serum from 28 homing pigeons (collected for another purpose) were tested for neutralizing antibodies to the W.E.E. virus. The results of these tests will be discussed later.

Two months after the collection of the tropical bird mites which were found to be infected with the W.E.E. virus, the E.E.E. virus was recovered from a horse brain submitted to us from Jefferson County, Texas, where a widespread epizootic was occurring, indicating that both viruses were active in Texas at about the same time. This extensive outbreak, which occurred in the Gulf States area and involved several counties surrounding the Beaumont, Texas area, afforded an excellent opportunity for continuation of our epidemiological investigations. Despite the extent of this outbreak which involved several thousand horses with over 4,000 deaths and at least 17 human cases of which 9 died in the Louisiana-Texas area, all attempts to demonstrate a virus in chicken mites and fowl ticks collected in the epidemic area were negative.

Another field survey was conducted at the time of a small outbreak of encephalitis in the Tarrant-Dallas Counties area in the fall of 1948. All attempts to demonstrate a virus in 6 pools of chicken mites, and 3 pools of fowl ticks, were negative. No serum specimens were collected at this time. No virus could be demonstrated in 9 pools of tropical bird mites which were collected the previous spring in the same area.

A summary of the attempts to recover a neurotropic virus from ectoparasites collected during the periodic surveys conducted in Texas between 1944 and 1948 is presented in Table 2.

RESULTS OF SEROLOGICAL TESTS ON SERUM SPECIMENS FROM AVIAN SPECIES

A summary of the results of neutralization tests on blood serums from 163 chickens (*Gallus domesticus*), 20 English sparrows (*Passer domesticus*), and 28 homing pigeons (*Columbia livia*) collected during periodic surveys is presented in Table 3. Fifty-four of the specimens were obtained from chickens less than one year of age; the remaining 109 specimens were from chickens hatched the pre-

TABLE 2

Results of attempts to recover the Western equine virus from ectoparasites collected during periodic surveys in Texas

Year	Equine Encephalomyelitis in Texas*			Ectoparasites Tested			Strains of WEE Isolated
	No. of mules and horses	No. of animals affected	Cases per 1,000 mules and horses	Source	Species	Estimated number tested	
1944	513,421	680	1.5	Chicken houses	*Dermanyssus gallinae* (De Geer)	5,000 (21)†	3‡
					Eomenacanthus strimineus (Nitzsch) (?)	4,000 (9)	0
					Argus persicus (Oken)	400 (6)	0
1945	139,959	38	0.3	Chicken houses	*Dermanyssus gallinae* (De Geer)	>6,000 (16)	0
					Argus persicus (Oken)	500 (7)	0
1946	216,504	137	0.6	Chicken houses	*Dermanyssus gallinae* (De Geer)	3,500 (13)	2 (?)§
					Argus persicus (Oken)	300 (6)	0
1947	316,321	685	2.3	English sparrow nests	*Liponyssus bursa* (Berlese)	7,000 (36)	5
				Chicken houses	*Dermanyssus gallinae* (De Geer)	3,000 (13)	0
				Chicken houses	*Argus persicus* (Oken)	500 (8)	0
				Wood rat burrows	*Triatoma sanguisuga* (Le Conte)	100 (5)	0
1948	127,177	78	0.7	English sparrow nests	*Liponyssus bursa* (Berlese)	2,000 (9)	0
				Chicken houses	*Dermanyssus gallinae* (De Geer)	1,500 (6)	0
				Chicken houses	*Argus persicus* (Oken)	200 (3)	0

* Information provided by Dr. L. T. Giltner, Bureau of Animal Industry, U. S. Department of Agriculture.

† Figure in parenthesis indicates number of pools tested.

‡ Two of these were from the same ranch.

§ Filtrable agent recovered but remained unidentified.

TABLE 3

Neutralizing antibodies for WEE in serum specimens from avian species tested during periodic surveys in Texas

Year	Avian Species Tested	Positive	Questionable	Negative
1944	Chickens			
	Old	5 (31.3%)*	2	11
	Young	2 (18.1%)	1	9
1945	Chickens			
	Old	9 (40.9%)	2	13
	Young	2 (13.3%)	1	13
1946	Chickens			
	Old	6 (23.1%)	5	20
	Young	0	2	12
1947	Chickens			
	Old	4 (12.5%)	4	28
	Young	0	1	11
	English sparrows	0	1	13
	Homing pigeons	2 (7.1%)	0	26
1948	English sparrows	0	0	6

* In computing percentages sera giving questionable reactions were omitted.

vious year or before. No attempt was made to estimate the age of the English sparrows or homing pigeons.

During the survey conducted in 1944, serum specimens were collected from 30 chickens; 12 were less than one year of age and the remaining 18 were hatched the previous year or prior to that time. Only 2 (18.1%) of the specimens from the young chickens contained neutralizing antibodies for the W.E.E. virus, while 5 (31.3%) of the 18 specimens from older chickens contained antibodies. In 3 instances the results were questionable. Three of 7 specimens from old chickens (none less than one year of age were available for study) on the Bennett ranch, where virus-infected chicken mites were collected, contained neutralizing antibodies for the W.E.E. virus. Only 4 specimens from old chickens on the farm where the fatal human case had occurred were obtained. No antibodies for the W.E.E. virus could be demonstrated in any of these specimens. No serum specimens were obtained in the area where the third pool of infected chicken mites was collected.

The next year (1945) serum was obtained from 40 chickens. When these were tested for neutralizing antibodies to the W.E.E. virus it became evident that a large proportion of those chickens which had lived through the previous epidemic season had antibodies. Nine (40.9%) of 24 serum specimens from old chickens contained neutralizing antibodies and in 2 additional instances the results were equivocal. Only 2 or 13.3% of the specimens from chickens hatched during the previous 6 months (a non-epidemic period) contained antibodies; in one addi-

tional case the results were questionable. During 1946, a non-epidemic season, 45 serum specimens from chickens were obtained. Only 6 or 23.1 % of the specimens from older chickens showed antibodies to the W.E.E. virus and 5 yielded equivocal results. None of the specimens from the young chickens contained demonstrable antibody. Storage for over one year in the CO_2 cabinet may have influenced the results of these tests.

More extensive field studies were conducted in 1947, an epidemic year, at a time when we were searching for a vector among avian blood-sucking ectoparasites other than those which parasitize chickens. Only one serum specimen from 12 chickens which had hatched during the previous 6 months showed antibodies and in this instance the results were questionable. Only 4 (12.5 %) of the specimens obtained from older chickens contained demonstrable antibodies. Because of the small amounts of serum obtainable from the English sparrows tested, these specimens were analyzed in pools. None of 5 pools of serum from 14 English sparrows contained antibody against the W.E.E. virus. Two (7.1 %) of the serum specimens from homing pigeons collected for another purpose* contained antibodies for the W.E.E. virus. During the spring of the following year only 6 specimens were obtained from English sparrows; none contained detectable amounts of antibody for the W.E.E. virus.

ATTEMPTS AT EXPERIMENTAL TRANSMISSION OF WESTERN EQUINE ENCEPHALOMYELITIS BY MEANS OF THE CHICKEN MITE, *DERMANYSSUS GALLINAE*

Soon after the W.E.E. virus was demonstrated in chicken mites (*Dermanyssus gallinae*) in 1944, experiments were undertaken to determine the role of this theoretically promising arthropod vector in the dissemination and perpetuation of this viral agent. Experiments designed to demonstrate a fowl-mite-fowl cycle, and attempts to establish transovarian passage of the virus in the chicken mite were undertaken. In view of the fact that detailed reports of the characteristics of the viremia in chickens produced by the subcutaneous injection of the W.E.E. virus were not available in the literature at the time these studies were initiated, it was necessary to devise experiments to supply this information which is so vital to any attempt at transmission of the disease by blood-sucking vectors. Since that time, however, Hammon, Reeves and Sather (1951) have reported in detail the results of their studies on viremia in experimentally infected birds to which they had made reference only in general terms in earlier publications.

Experiments were conducted to compare the ability of various strains of W.E.E. virus to produce a viremia in young chickens and to determine the duration of such a viremia. Four virus strains were used in these experiments: the "Olitsky", "Howitt", Bennett mite and Ridgeway strains. Incubator-hatched chicks were transferred to the laboratory where exposure to blood-sucking arthropods was minimal. The age of the chicks varied from 33 to 47 days. Chicks were inoculated subcutaneously with virus suspension and were then bled at designated intervals from either a wing or jugular vein by means of a tuberculin syringe containing a

* The authors are grateful to Mr. Earl W. Sudderth, Dallas County Sanitary Engineer, for assistance with these collections.

dilute solution of heparin in broth. Samples from each chicken were immediately inoculated intracerebrally in 3-week-old white Swiss mice. Animals were observed during a period of 3 weeks for central nervous system symptoms.

In the initial experiments it appeared that the recently isolated strains (Ridgeway and Bennett strains) produced a viremia which persisted longer than that produced with the laboratory-adapted strains. For this reason the Bennett mite strain was used in most of the subsequent experiments. Since much of the qualitative data accumulated in this laboratory is in agreement with that reported by Hammon and Reeves (1946) detailed protocols will not be presented. An analysis of a typical set of experiments showed that virus could be demonstrated most consistently in the blood of chickens between 8 and 32 hours following subcutaneous inoculation. Only 2 of 23 chickens failed to show virus in the bloodstream at any time; in 5 instances virus was detected only once. Other experiments designed to determine the effect of the size of the virus inoculum on the persistence of detectable viremia in chickens have been conducted. These experiments have shown that the time of appearance and the duration of the viremia may vary considerably from chicken to chicken even under identical experimental conditions. In addition, the extent of the viremia in some experiments seemed to be influenced by the virus dose used for infecting the birds. These facts had to be taken into consideration in designing experiments to demonstrate experimental transmission of the W.E.E. virus by means of the chicken mite. Under those conditions in which it was possible to produce a viremia in chickens it was then necessary to determine whether actual proliferation of the virus had occurred. In contrast with the experiences reported by Hammon and Reeves (1946), evidence for virus proliferation in the bloodstream of chickens receiving subcutaneous inoculation of the Bennett mite virus was only suggestive. Whether or not this was due to the fact that a "brain-adapted" rather than a "blood-adapted" virus strain was used in these experiments was not determined.

During the same period of time that studies were being performed on the characteristics of the viremia in young chickens, attempts to demonstrate transmission of the W.E.E. virus by means of the chicken mite were in progress. Later, however, a procedure was established which would include several internal checks upon each attempt and would yield reliable results. Hatchery chicks reared under the same conditions previously described were used. The ages varied from 1 to 6 weeks. The laboratory adapted "Howitt" strain and the Bennett mite virus in its second mouse passage were used in these experiments. Virus dilutions were made in nutrient broth and the inoculation of chickens was made through the subcutaneous route in all cases. Three-week-old mice were inoculated intracerebrally to check for viremia in the inoculated or exposed chicks.

In general the following procedure was used in the transmission experiments. Virus-free starved chicken mites from the stock colony were used. Chicks were inoculated with the dilution of virus selected. A blood sample was obtained from each chick immediately prior to the period during which the mites were introduced into the chamber. The mites were allowed to feed for 12 to 24 hours, after

which the chick was again bled. The subsequent course depended upon whether or not virus was present in the peripheral bloodstream during the time when the mites were allowed to feed. If no virus was detected by mouse inoculation, the procedure was repeated. If viremia had occurred, a susceptible chick was exposed to the mites which had been allowed to feed on the infected chick. Serum specimens were obtained before and after exposure to the mites which were allowed to feed for not more than 12 hours. In addition, blood samples obtained 12, 24, and 36 hours after removal of the mite colony from the chick were tested for virus by mouse inoculation. A blood specimen obtained 15 days after the mites had been removed was tested for neutralizing antibodies. Samples of mites used in the attempted transmission experiment were tested periodically for virus, and in a number of instances mites from these colonies were allowed to feed on young susceptible chicks.

Since these experiments were initiated in 1945, a total of 16 attempts at the transmission of W.E.E. by means of the chicken mite have been made. The virus inoculum was varied in some experiments and the period of mite feeding ranged from 8 to 67 hours following inoculation of the chicks. The "Howitt" strain was used in 4 of these experiments and the Bennett mite strain was used in the remaining 12. Viremia was demonstrated in the chicks at the time the mites fed in only 5 of the 16 attempts, and in only 2 instances was it possible to demonstrate virus in the engorged mites.* At no time was it possible to transmit the infection to young normal chicks by permitting "infected" mites to feed on them. None of these chicks developed neutralizing antibodies. All attempts to demonstrate evidence of transovarian passage through the identification of virus in protonymphs were negative. While these studies were in progress it was encouraging to learn that Hammon and Reeves (1946) also showed that mites neither became infected nor infective after permitting them to feed on inoculated birds which had developed a viremia.

DISCUSSION

The demonstration of the equine encephalomyelitis viruses in chicken and wild bird mites suggested that a reservoir for these agents had been found. The low infection rate and the futile attempts by us and others (Reeves *et al.*, 1955; Chamberlain and Sikes, 1955) to demonstrate experimental transmission in fowl by means of the mite necessitate continued search for other means for the perpetuation of these viruses from year to year. It is estimated that over 30,000 arthropods have been tested by us for natural infection with neurotropic viruses since these studies were initiated. Except for the unidentified agent recovered from two pools of chicken mites collected in 1946, the demonstration of the W.E.E. virus in 3 of 21 pools of chicken mites tested in 1944 could not be repeated. In studies conducted during subsequent years 48 pools of mites were tested for virus with uniformly negative results. Similarly, the recovery of this

* It now appears that viremia might have been demonstrated more frequently if ½-day-old chicks rather than young mice had been used for testing for virus (Chamberlain, Sikes, and Kissling 1954).

virus from 5 of 36 pools of tropical bird mites, *Liponyssus bursa*, tested in 1947 could not be duplicated in limited tests conducted the following year. While the W.E.E. virus was isolated by us in relatively few instances it would appear that the proportion of infected mites observed in these studies was considerably higher than that observed by Hammon and his associates (1951). The relative infrequency with which one finds antibodies for the W.E.E. virus in chickens in Texas might account for the few "chance" isolations of the equine virus from mites. It is conceivable that following a natural exposure an occasional chicken or wild bird may have persistent viremia and no detectable antibody response. The finding of virus in mites may be significant only as an indication that the mites have recently fed on chickens or wild birds with viremia.

Neither we nor Reeves and his colleagues (1955) have been able to produce experimental transmission of the W.E.E. virus by means of the mite.* In the single instance in which Chamberlain and Sikes (1955) observed transmission of this virus to the chicken by means of the bite of infected chicken mites, *Dermanyssus gallinae*, virus was detected in the blood of the chick, sacrificed after 24 hours exposure to the mites, by the subcutaneous inoculation into chicks but not by the intracerebral inoculation into mice. It is quite possible that experimental transmission might have been demonstrated by us if the more sensitive chick method had been used for the detection of virus. Because of the short duration of the viremia following experimental infection together with the fact that antibodies generally appear between 10–15 days after inoculation, it is unlikely that the chicken serves as a natural reservoir for this virus. Domestic fowl, however, may serve as sources of infection for mosquitoes or other blood-sucking ectoparasites for short periods of time after an infecting bite. Wild birds, on the other hand, are more likely virus reservoirs because of the longer persistence of virus in the bloodstream following experimental infection (Hammon *et al.*, 1951). All attempts to demonstrate transovarian passage of the W.E.E. virus in the chicken mite have been negative. The studies of Chamberlain and Sikes (1955) have also shown that fowl mites may be relatively unimportant as mechanisms of perpetuation of this virus in nature. Because of the infrequency with which these arachnids become infected and transmit infection by bite or by transovarian passage in the laboratory it appears likely that this is also true under natural conditions. After numerous attempts they succeeded in one instance in transmitting infection with the Western equine virus to young chicks through the bite of infected chicken mites. Also, on one occasion transovarian passage of this virus was demonstrated in *B. sylviarum* protonymphs.

The W.E.E. virus strain used probably had little to do with the outcome of these experiments. In similar studies conducted in three different laboratories, seven strains of W.E.E. virus were used; the four referred to in this report; an agent isolated from *Culiseta melanura* employed by Chamberlain and Sikes (1955); strain F 199 recovered from mosquitoes and BFS 1034 recovered from wild bird mites used by Reeves and his associates (1955).

* In a limited number of experiments, Day was unable to demonstrate experimental transmission of the Murray Valley Encephalitis virus by means of the chicken mite, *Dermanyssus gallinae*.

Although certain mosquitoes, *Culex pipiens* and *Anopheles maculipennis freeborni*, have been found to be naturally infected with the W.E.E. virus, their role as vectors of this disease has been questioned because experimental transmission could not be effected (Hammon and Reeves, 1946). The same reasoning applies in assessing the role of mites as possible vectors or reservoirs of Western equine encephalomyelitis. It must be concluded from this summary of the results of field and laboratory studies that although mites which parasitize wild birds and domestic fowl may harbor encephalitis viruses, they play no essential role in the natural history of the diseases caused by these agents.

SUMMARY

The present report summarizes the results of field and laboratory studies designed to determine the role of mites as possible vectors or reservoirs of Western equine encephalomyelitis. It is concluded from these studies that although occasionally chicken mites or wild bird mites are found to be naturally infected with this virus they play no essential role in the epidemiology of the disease. The demonstration of the Western equine virus in chicken mites or in wild bird mites may be significant only as an indication that they have recently fed on infected chickens or wild birds.

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MORAY EEL POISONING AND A PRELIMINARY REPORT ON THE ACTION OF THE TOXIN[1]

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Previous studies on ichthyosarcotoxism have been divided into two distinct categories, viz., clinical reports, which for the most part are not accompanied by laboratory findings, and laboratory analyses of fishes which have been screened for the presence of toxins in an epidemiological survey by this and other laboratories. It has been assumed without direct proof that the action and properties of the poison as tested in our laboratory are the same as those observed in clinical cases. The present study tends to confirm our conclusion that we are dealing with a single clinical entity, and provides a sound basis for laboratory investigation of the nature of the toxin or toxins involved. The present study was stimulated by an outbreak of moray eel poisoning among six Filipino laborers at Kwajalein, Marshall Islands, on March 21, 1953. A sample of the toxic fish was frozen, shipped to this laboratory, and tested as exhaustively as the material would permit.

Khlentzos (1950) has written the only known previous paper concerning the clinical effects of moray eel or gymnothorax poisoning. More has been written on laboratory studies of the toxin. In 1888 Mosso reported that the serum of the European moray eel, *Gymnothorax helena* (Linnaeus) contained a substance which, when injected parenterally into certain mammals, resulted in hemolysis, decreased sensitivity to pain, hypersalivation, respiratory distress, clonic convulsions, paralysis and death. Kopaczewski (1917, 1918) later found that the toxic principle in moray eel serum was destroyed by heating at 56°C. for 15 minutes and that radiation by ultraviolet light of wave length 224 to 300 mu destroyed the toxin, but freezing and desiccation had no deleterious effects upon it. The toxin was said to be inactivated by gastric enzymes and had hemolytic properties. Moreover, the serum had a bacteriolytic action against *Staphylococcus aureus*. Comprehensive reviews of the toxic properties of moray eel serum have been given by Phisalix (1922), and Pawlowsky (1927). None of the preceding authors discuss the edibility of moray eels.

Jordan (1929), Matsuo (1934) and Yasukawa (1950) have reported that certain species of moray eels of the genus *Gymnothorax* are considered to be poisonous to eat in certain areas in the tropical Pacific. Hiyama (1950) appears to have been the first to demonstrate the toxicity of *Gymnothorax javanicus* (Bleeker),[2] *G. meleagris* (Shaw and Nodder), *G. pictus* (Ahl) and *G. undulatus*

[1] This investigation was supported by a research grant from the Division of Research Grants and Fellowships, National Institutes of Health, Public Health Service, and a contract from the Office of Naval Research, Department of the Navy (Contract No. NONR 205-00).

[2] This species is mistakenly identified in Hiyama's work as *G. flavimarginatus*. If the illustration is correct then the species is *G. javanicus*.

(Lacépède). Recent screening studies in our laboratory have shown that *G. buroensis* (Bleeker), *G. flavimarginatus* and *G. petelli* (Bleeker) are also toxic.

The toxicity of moray eels, as in the case of most poisonous fishes, is not constant. A species that is toxic in one locality may be edible in another. The factors governing the toxicity of fishes are not understood at present, but epidemiological studies conducted thus far by Halstead and Bunker (1953) indicate that fishes become poisonous as a result of their feeding habits. The state of freshness has no bearing on the production or the virulence of the toxin since putrefaction is an incidental factor in the disease.

EPIDEMIOLOGY AND CLINICAL CHARACTERISTICS

The eel was captured in the South Pass of Kwajalein Atoll and was described as having a greenish head, dark brown to black body, speckled with light markings and having a green streak along the back. The color description appears to be that of *G. flavimarginatus*, but additional data would be needed before a conclusive identification could be rendered.

The eel was kept alive until it was prepared for cooking on March 21. The fish was prepared by cutting it into slices and boiling it with some seasoning. The eel was consumed while it was warm. The onset of symptoms occurred in two hours in five of the patients and seven hours in one case, after the ingestion of the fish. The victims were sent to the local Naval Station Infirmary for hospitalization. The victims developed marked neurological symptoms consisting of generalized numbness, heaviness in the legs, ataxia, "dizziness" and a feeling of tightness of the face and lips. Some of the patients had severe retching with vomiting, but no diarrhea. Generalized malaise was said to be pronounced.

Upon examination, four to five hours after the initial onset of symptoms, the victims appeared acutely ill, anxious and panicky, but were alert and oriented. Severe ataxia, marked difficulty in voluntary movements of the facial muscles and extremities, hyporeflexia and carpal spasm were present. Chvostek's and Rhomberg's signs were positive. Gag reflex, corneal reflex and ocular movements were normal. Sensory examination was normal to deep and superficial pain. There were no meningeal signs. Abdomen was soft and non-tender, but marked borborygmus was heard. Respiratory rate, blood pressure and pulse rate were within normal limits. Data regarding the laboratory findings were not available.

Upon admission the patients were given a saline purge. Previous vomiting eliminated the necessity of gastric lavage. Intravenous injections of 1.5 to 2.5 gm. of 10 per cent calcium gluconate and 10 per cent calcium chloride were administered. Relief was prompt in five of the patients. Three hours later one of the patients had a relapse, but was promptly relieved with an additional injection of 2.0 gm. of calcium gluconate.

The sixth patient appeared to be more severely intoxicated than the others. Ataxia with almost complete loss of control of the muscles of the back and extremities, glottal and carpopedal spasm were pronounced. The patient did not respond to calcium therapy and became irrational. Sixteen hours later he developed a grand mal seizure and then lapsed into coma. He was subsequently transferred to the Queen's Hospital in Honolulu. Upon hospitalization on March

25, 1953, the patient was found to be deeply comatose, having intermittent episodes of yawning and grinding of the teeth. There was no pupillary reaction to light, but there was spasmodic constriction and dilatation of the pupils. Corneal reflexes were absent. Chvostek's sign was absent. Breathing was by accessory respiratory muscles. There was no response to painful stimuli. All deep tendon reflexes, Babinski's sign, ankle and patellar clonus were negative. There was a moderate spasticity of the upper extremities, generalized weakness of extensor muscles, bilateral foot drop with both feet internally rotated and a generalized hypotonicity of all muscles with the exception of the upper extremities.

The laboratory findings were not noteworthy except for the CO_2 combining power which was 16.5mM per liter. The patient was in a critical condition during the 21 days of hospitalization. His rectal temperature varied between 100°–102°F., pulse 130–140, respiration 20–24. After the third day the patient developed Cheyne-Stokes respiration and was placed in a respirator where he remained for the duration of his hospital stay. He continued to be comatose and died 25 days after eating the meat. Treatment was largely symptomatic, consisting of parenteral feedings, penicillin, aminophylline, and caffeine sodium benzoate. (The authors are indebted to Captain M. J. Hantover, Commanders A.N. Chaffin and W. J. Hall, USN, and Drs. H. L. Arnold and G. H. Mills for the preceding clinical data.)

LABORATORY STUDIES

Specimen material consisted of frozen transverse sections of eel and eel broth making a total of about one liter of material which was secured from the Filipino victims by Commander W. J. Hall and shipped via the U. S. Fish and Wildlife Service in Honolulu. The material was kept frozen until used for testing. At that time bacteriological cultures were made but revealed only sparse growth of a nonpathogenic coliform colony.

An oral LD_{50} of the broth for the mice was calculated on 30 mice by the method of Litchfield and Wilcoxon (1949). The LD_{50} was 1.35 ml. of the broth per mouse or 0.237 gm. solid material. The 19/20 confidence limits were from 0.039 to 0.404 gm. solid material per mouse.

Water or acid methanol (pH 4.0 with acetic acid) proved to be the most efficient of the extractants tested. Injection of water solutions of either of these extracts produced essentially the same picture as given by the oral administration of the broth though parenteral administration gave almost immediate response. The symptoms in mice were, in general, rapid in onset and pronounced, consisting of stretching, piloerection and respiratory stimulation; later the mice developed lacrimation and concretions of the conjunctiva, diarrhea, hypoactivity, ataxia and respiratory distress, and ending in clonic convulsions, respiratory arrest and death. Cardiac inhibition could not be detected. Results in rats were similar. Only one rabbit was injected because of our limited supply of material, and it followed the same general picture, dying in clonic convulsion.

Anesthesia or calcium ion therapy did not seem to alter the course of the

poisoning, though we do not consider our data conclusive at this time. The benefit obtained from calcium therapy in the clinical studies could be referable to a direct alleviation of a hypocalcemia or a treatment of the acidosis measured in the low CO_2 combining power. The hyperventilation and shock associated with the acute phases of the poisoning in laboratory animals suggest a profound alteration of electrolyte balance and blood pH which would be rapidly amenable to calcium.

DISCUSSION

The evidence presented establishes that the clinical problem of fish poisoning in man is the same as that studied in the laboratory and does not appear species specific. The finding of toxicity in the laboratory animal is not an artifact of the shipment and extraction procedures. The poison has potent central nervous system effects to the level of a convulsant. The vomiting in man and the hypersalivation and diarrhea in mice suggest a parasympathetic stimulation.

The chemical nature of the poison is only partially defined by this study. It was soluble in water and acid methanol and resisted destruction by the pH and temperature changes of the various extraction procedures. It was similar to a number of extracts from other species in that it is dialysable (Halstead and Ralls, 1954).

SUMMARY

A comparison between the clinical picture of moray eel poisoning in man and the effects of the poison on laboratory animals indicates that they are the same. The toxin is soluble in water and acid methanol and is stable to the extraction procedures investigated. It is dialysable. Further identification of the chemical nature of the toxin was prevented by the limited supply of raw material.

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DISPERSION OF MARKED AMERICAN COCKROACHES FROM SEWER MANHOLES IN PHOENIX, ARIZONA

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The association of cockroaches with filth is common knowledge, and the ability of these insects to carry a wide variety of microorganisms has been demonstrated repeatedly by early workers (Herms, 1950). More recently it has been found that roaches trapped in sewer manholes were infected with a number of species of *Salmonella* bacteria known to be pathogenic to man (Bitter and Williams, 1949; Anon., 1953). Other recent studies have shown three species of roaches collected from sewer environments in widely separated areas of the country to be infected with poliomyelitis virus (Syverton, *et al.* 1952). The cockroach is, therefore, a potential vector of at least several filth diseases, and its capacity to transmit these diseases needs to be evaluated under varied field conditions.

The movements of cockroaches from a source of contamination to an area of human contact constitute an important phase of such an evaluation. In previous dispersion studies in Phoenix, Arizona, and Tyler, Texas, where the American cockroach (*Periplaneta americana* (L.)) thrived in large numbers in sewer manholes, from 1,000 to 6,500 marked extraneous roaches were superimposed on normal populations that were small in comparison, and the dispersion patterns were determined by recapturing released individuals. The Phoenix study indicated virtually no movement, while that at Tyler showed widespread roach dispersion from manholes into adjacent homes (Schoof and Siverly, 1954; Anon., 1953). The present study at Phoenix was undertaken in July, 1953, to determine the nature of roach dispersal from sewer manholes during the hot summer months from both a normal undisturbed population and one drastically disturbed by the superimposition of a large number of roaches caught elsewhere.

METHODS AND PROCEDURES

The American cockroach, the only species observed to inhabit sewer manholes in the Phoenix area, was used for these studies. Roaches were collected for marking purposes and were recaptured, after their release, with a trap consisting of a one-quart, wide-mouthed mason jar having an inward-directed, plastic screen cone held in place by a standard cover ring. Ripe bananas served as bait. Carbon dioxide gas was used as an anesthetic to facilitate handling the roaches.

In the first of three dispersal tests, roaches were marked by placing the trapped individuals in a garbage pail, ringed near the top with petrolatum to prevent their escape, and then spraying them with a lemon-yellow, rubber-base paint dispensed from a small, vibrator-type sprayer. A random sample of 20 roaches withheld for laboratory observations showed all adults to have retained their markings satisfactorily one week after treatment, while the nymphs had lost nearly all of theirs. Three weeks following the treatment, 50 per cent of the adults

were still well marked. Paint spots on the elytra were the most persistent. Spots on other areas seemed to be more readily cleaned off and, when touched with a probe, often scaled off easily.

The site of this first release was a sewer manhole in an alley of a middle-class residential block. The nearest manholes on the same sewer lateral were 608 feet downstream and 460 feet upstream. Three hundred roaches, about 75 per cent of the existent manhole population, were trapped during the night of July 28, marked the next morning, and then returned directly to the manhole.

Traps were placed in 23 yard sites next to garbage cans or refuse piles adjacent to the alley, in 3 shed sites, and in 19 residential sites, usually in the kitchen or bathroom. Traps were usually replaced daily during the 5-day trapping period. The two neighboring manholes, though not trapped, were checked daily for the presence of painted roaches.

In the second and third experiments, the roaches were tagged with radioactive phosphorus (P^{32}). As before, the roaches were placed in a garbage can and sprayed with the marking agent having casein as an adhesive. About 100 cc. of a 5 per cent aqueous casein solution containing ten microcuries of P^{32}/cc. were used to treat each of the two groups of roaches. Standard procedures to minimize radiation hazards were observed during these operations. In addition, a clear plastic cover with a central nozzle opening was placed over the garbage can to prevent the scattering of radioactive mist. The authors appreciate the assistance of Dr. H. F. Schoof, Dr. R. W. Fay, and Mr. Jens A. Jensen, Communicable Disease Center personnel, who made unpublished information on this marking technique available and assisted otherwise with this phase of the experiments.

Most of these roaches, whether retained in the laboratory or recaptured after their release into manholes, exhibited 500 to 2,000 counts per minute above background as determined by a beta-gamma radio-activity monitor. Some ranged as high as 6,000 cpm and others as low as 20 cpm above background. The latter may have become radioactive secondarily through cannibalism; however, these could not be differentiated from the roaches receiving a low-intensity initial tag. A few of the roaches were returned to the manhole in a moribund condition, and these may have produced this low-intensity tag when eaten by non-tagged roaches. The dispersion patterns are no less meaningful because of this uncertain source of radioactivity; for such individuals were few, and secondary marking most likely occurred in the release manholes.

These two experiments were carried out in a well-sanitated, low-rent housing project arranged in quadrangles, each containing 20–24 apartments. The area was well supplied with shrubbery and lawn areas. A single manhole and its associated laterals serviced each quadrangle. In the study quadrangles, traps were placed around the yards of each apartment, usually adjacent to the garbage can. Additional traps were located in such protected habitats as tall grass, vines or bushes. Most residents allowed traps to be placed in their apartments, usually in the kitchens or pantries. Areas peripheral to the study quadrangles were not trapped. Traps were usually replaced daily, and both upstream and downstream manholes were trapped continuously during the course of the experiments. This intensive trapping was maintained for 15 days following each release.

The release-site manhole in the second experiment was so located that a manhole was 165 feet upstream at the head of the sewer line and 5 manholes were downstream 185, 350, 535, 700 and 839 feet from it, the last being the juncture with the main sewer line. Several days previous to the second experimental release, approximately 1,200 roaches were trapped from several manholes outside the housing project. They were held in the laboratory until August 6 when they were tagged and superimposed on the resident population of 300 roaches in the release-site manhole.

The release site for the third experiment was located on the opposite side of the same housing project area to prevent any intermingling of roaches from the two releases. This release-site manhole was situated at the upper end of a sewer line and 340 feet distant from the main line. An intermediate manhole existed half way between these points.

On August 10, the day prior to the release date, traps were placed in the release-site manhole. About 500 roaches, almost all of the visible roach population in this manhole, were captured by the next morning with the use of traps supplemented by a vacuum cleaner with a padded attachment. These were tagged in the laboratory and immediately returned to their native manhole; no foreign roaches were superimposed on this population.

RESULTS

In the first release of marked cockroaches, no painted individuals were recovered during five days of intensive trapping, although unmarked roaches were caught in yards and homes (Table 1). No marked individuals were observed in neighboring manholes. Observations in the vicinity of the release site during two evenings in this period failed to reveal any roach movements, and no roaches were caught in traps set over holes in the manhole cover.

The ratio of marked to unmarked roaches in the release-site manhole progressively diminished from 3:1 at the time of release to 1:4 seven days later. While the loss of markings, death and/or cannibalism, or a gradual dispersal could explain this change, it is probably that the last was most important. Although nymphs readily lost their markings, adults retained theirs and the ratio of marked to unmarked adults decreased. General observations indicated that death or cannibalism removed only a small number of roaches during the short test period. A gradual dispersal of marked individuals from the manhole into the sewer lines, appurtenant laterals, and house connections seemed the most adequate explanation for the declining number of marked roaches, since none was recovered or seen above ground or in neighboring manholes.

A few marked roaches were observed during the next three months in the release-site manhole. Two months after the original release, a marked female roach was captured in a house about 125 feet in sewer-line distance from the release site. This was the only tagged individual found outside the release-site manhole despite intensive searching and trapping and the enlistment of aid from nearby residents.

In the second release experiment, where 1,200 foreign radioactive roaches were superimposed on a native manhole population of 300, 71 tagged individuals

TABLE 1

Summary of trapping results in three cockroach dispersion experiments

	House traps	Yard traps	Manhole traps	Total
Release 1 (300 roaches)				
Number traps	19	26*	—	45
Number trap nights†	88	125	—	213
Total number roaches caught	19	4	—	23
No. marked roaches recaptured	0‡	0	—	0
Release 11 (1,200 roaches)				
Number traps	21	30	6	57
Number trap nights†	300	443	84	827
Total number roaches caught	981	188	5,830	6,999
No. marked roaches recaptured	1	27	43	71
Release 111 (500 roaches)				
Number traps	20	24	2	46
Number trap nights†	260	345	30	635
Total number roaches caught	191	61	1,350	1,602
No. marked roaches recaptured	0	1	3	4

* Includes 3 traps in sheds.

† A trap night is one trap operating for one night.

‡ One marked roach was recaptured in a house by the residents two months after the release.

were recaptured during the 15 days following their release into the manhole. One was caught in a kitchen 80 feet from the release site and 27 in five yard traps at distances of 95, 60, 50, 50, and 15 feet from the release site. However, 22 of these 27 were caught at the same site, a small cavity in the ground adjacent to an apartment wall and 50 feet from the manhole. Excavation following the completion of the experiment showed that the hole in which the trap had been placed was confluent with an abandoned sewer, probably a house connection, which in turn was connected to the sewer running through the project. Thus a roach could have readily reached this site from the release-site manhole by travelling 65 feet through sewer lines. While many of the roaches caught at this site undoubtedly followed this subterranean route, it is quite possible that some escaped from the release-site manhole through holes in the cover and arrived by an overland route. It is possible, too, that some of the roaches caught in other yard and home traps may have dispersed secondarily from this depression rather than by the more obvious route through the manhole cover. This strongly suggests that sewer lines similar to house connections may be a favored habitat of the American cockroach.

In addition, 43 tagged roaches were trapped in manholes near the release site: 8 in the manhole upstream which is the origin of this sewer line, 33 in the manhole immediately downstream, and 2 in the second manhole downstream. The other manholes located further downstream remained negative for marked individuals. Thus the maximum distances between the release site and the points of recapture for all 71 roaches in the second release were 80 feet into a home, 95 feet overland into a yard trap, and 350 feet through sewers.

In the third release experiment where only the 500 roaches native to the manhole were tagged with P^{32}, four marked individuals were caught in 15 days of intensive trapping. One was recaptured in a yard trap 33 feet from the release site and three in the nearer neighboring manhole, 170 feet downstream.

DISCUSSION

Very little dispersion of cockroaches occurred in two experiments where roach population native to manholes were marked without disturbing or creating population pressures. However, in another experiment where the population balance was drastically upset by the superimposition of a group of foreign roaches four times the size of the resident population, considerable dispersion did occur to the extent that 5.9 per cent of the released roaches were recaptured at points outside of the release-site manhole. In contrast, the recovery of marked roaches was only 0.8 per cent in an experiment where no superimposition of foreign populations occurred.

These results support the general hypothesis that, under usual manhole conditions, cockroach populations exhibit little tendency to emigrate. Thus, while localized shifting and moving of the roaches from manholes into laterals probably occur regularly, more extensive travel from sewer laterals into yards, homes, and main lines is occasional and of comparatively short range. The population as a whole remains rather immobile except for the sporadic emigrations of a few individuals.

In situations, however, where strong population pressures are created, such as the crowding that results from the artificial superimposition of individuals or from the partial flooding of a sewer system, emigration can be expected to radiate from the center of positive population pressure. In contrast, areas with negative pressures may have a reverse influence on population movements and attract roaches into them. In the first experiment, neighboring manholes were not trapped so that this latter disruptive factor would not be involved in the test; but in the two experiments in the housing project, such trapping was done. Here considerable numbers of roaches were removed from neighboring manholes, but there was little indication that the negative pressures which developed had much effect on the results.

A previous experiment conducted in Phoenix (Schoof and Siverly, 1954) indicated virtually no movement of roaches during October when a total of 6,500 tagged individuals was superimposed on four manhole populations and only one roach was recaptured. Surrounding manholes were trapped intensively, and a few traps were placed in yards adjacent to the release sites. However, the manholes in the release area were 720 feet apart which is a far greater distance than an emigrating roach might be expected to travel if the present results be applicable. Thus it is probable that the dispersion which occurred was not adequately measured by the trapping procedures employed.

Reports of extensive roach movements from manholes into nearby homes have come from Texas (Anon., 1953). But here again groups of 1,000 individuals were superimposed on native populations. Such procedures would seem likely to

produce centers of strong population pressure at the release sites, and emigration would be the expected result.

The Phoenix experiments of 1953 quite conclusively indicated that only minor normal cockroach emigration from manholes into homes, yards and neighboring manholes occurred under the observed summer conditions. The results, however, do not preclude the possibility of large-scale roach movements under different climatic conditions, perhaps in the late spring. Further observations on this hypothesis are yet to be made.

However, the fact that roaches do move from sewer systems into homes, at least sporadically, makes them potential vectors of pathogenic enteric organisms. Their importance as potential vectors of intestinal diseases is probably increased by the constant arrival at their habitat of human fecal materials from distant sources that probably serve as roach food. Thus even with a very limited home range, it is possible for cockroaches to carry enteric pathogens from many distant sources to susceptible humans. In this way roaches may play a part in setting up new foci of enteric diseases.

Much more data are needed on the frequency with which roaches normally enter homes and on their modes of entry, such as swimming through water seals of plumbing fixtures, crawling through doors, windows or other openings, or entering in other ways now unsuspected. In addition, more epidemiologic data are needed to establish the capacity of cockroaches to transmit pathogenic enteric organisms.

SUMMARY

Movements of the American cockroach, *Periplaneta americana*, from sewer manholes in Phoenix, Arizona, during the summer of 1953 were studied by a trap, mark, release, and recapture technique using paint or radioactive phosphorus. In two experiments where only the resident manhole populations were marked, very limited emigration was observed; only 4 of 800 marked cockroaches being recovered by intensive trapping in yards, homes, and neighboring manholes. However, when 1200 marked cockroaches were superimposed on a resident manhole population of 300 roaches, 71 tagged individuals were recovered within 15 days: one in a home, 5 in yards, and 65 still in the sewer system at distances up to 350 feet from the release point. This suggests that a sudden increase in population over that of the carrying capacity of the environment may incite emigration from the center of pressure.

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THE CONTROL OF THE MONGOOSE IN PUERTO RICO

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The potential danger of rabid mongooses to the health and safety of the public in Puerto Rico prompted action by the Public Health Service to find an effective method of control for the Indian mongoose, *Herpestes auropunctatus*. Only 21 cases of rabies were diagnosed in dogs and farm animals in Puerto Rico during the period from 1911 to 1933 (Tierkel *et al.*, 1952). From 1923 to 1950, the island was considered one of the few rabies-free areas in the world. In March 1950 the present outbreak of rabies began on the island. Studies by the Commonwealth of Puerto Rico Department of Health and the Public Health Service indicated that the mongoose was the important reservoir and vector of rabies in Puerto Rico (Tierkel *et al.*, 1952). This was the first time rabies had been reported in the Indian mongoose. In South Africa, however, the yellow mongoose has been recognized as a vector of rabies since 1928 (Synman, 1940).

Mongooses are of further concern to public health officials because of the high incidence of infection in these animals with the human leptospiral organism (Yeager, 1952.)

The present recommended method of mongoose control is trapping, a procedure that is reasonably successful but time-consuming and expensive. The aim of this study was to find a chemical control method which would be more economical and effective than trapping.

DEVELOPMENT OF A CHEMICAL CONTROL METHOD

The biology of the mongoose was investigated to provide guidance in planning the control studies (Pimentel, in press). Concurrently with the investigations on the biology of the mongoose, tests were carried on to select a suitable bait and poison to use in subsequent field control experiments.

Bait selection. Since the mongoose is a carnivore, an economical meat-type bait was needed as a vehicle for the poison. Fish was found to be the cheapest bait in Puerto Rico. Fresh fish, under the prevailing tropical conditions, decomposed in about 2 days. Thus, to find a long-lasting bait, salted codfish, seared salted codfish, sun-dried desalted codfish, and sun-dried fresh fish were tested in the laboratory and in the field. The mongoose refused to eat the heavily salted baits. Both of the sun-dried fish baits were acceptable and remained so in the field for 2 weeks, if kept dry. Since sun-dried fresh fish was cheaper, it was used in subsequent tests.

Laboratory poisoning tests. Laboratory tests were made for the purpose of selecting a poison to use against the mongoose in field control experiments. The animals were maintained on ample amounts of fish before the tests began. Since bait preference tests showed that an adult mongoose consumed about 2 ounces of fish over a 24 hour period, the poison was spread over 1 ounce of fish to encourage consumption of all the poisoned bait.

Results with 54 test mongooses indicated they were naturally resistant to warfarin at the dosages and time periods tested. The daily dosages varied from 5 mg./kg. to 300 mg./kg. for periods of 3 to 14 days. Even at the extremely high daily dosages of 300 mg./kg. for a 5 day period only 2 of the 4 test animals died. To check whether the fish in the bait was acting as an antidote for the warfarin, canned dog food which contained no fish was fed in a similar manner with comparable results. Two mongooses fed a 600 mg./kg. dosage of red squill vomited the poison. Barium carbonate at an 80 mg./kg. dosage was not effective against 3 mongooses. Bait refusal was noticed with bitter-tasting strychnine at a 40 mg./kg. dosage. Because of the initial ineffectiveness of these poisons, no further tests were made with them.

ANTU, zinc phosphide, thallium sulfate, microfine arsenic trioxide, and sodium fluoroacetate ("1080") were tested against 166 mongooses. Poison bait refusal was noted with the higher concentrations of ANTU, zinc phosphide, and arsenic trioxide, but not with thallium sulfate and "1080". The majority of the animals fed these five toxicants in the higher concentrations vomited the poison bait. The vomiting probably explains the erratic results, for if the mongoose vomited immediately after ingesting the poison bait there was a good chance of the animal surviving. When "1080" was administered at a dosage of 80 mg./kg. all the test animals were killed. This poison, however, was considered too dangerous for use in the field.

As a check on the methods and materials used, 2 white rats were poisoned with each of the 9 toxicants, using the same fish bait. In each test, a dosage which would produce an $LD_{90\text{-}100}$ was used. The results were comparable to those obtained by other workers, indicating that the techniques of administering the poisons to the mongoose did not influence the effectiveness of the various poisons.

Ground glass (maximum particle size 2 mm.) was fed to mongooses. Only one of the 4 test animals died. Numerous lesions were found in the small intestine which may have accounted for its death.

A 60 mg./kg. dosage of thallium sulfate was successful when fed three times, either daily, every other day, every third day, or every fourth day. This dosage of thallium sulfate was used in the field control experiments.

FIELD CONTROL EXPERIMENTS

The poison mixture was prepared by thoroughly mixing 1 part thallium sulfate, 1 part powdered green dye, and 8 parts white flour. The bait used was fresh fish sun-dried for 8 to 10 hours. Since the mongoose consumes about 2 ounces of food per day, the poison mixture was spread over a 2-ounce piece of fish before sun-drying. This quantity of bait reduced the chances of the mongoose eating more than one piece of poisoned bait during one day, since in laboratory tests it was noted that mongooses frequently vomited daily dosages exceeding 60 mg./kg. of thallium sulfate.

Since the average weight of the mongoose is about half a kilogram, a quantity of mixture equivalent to 30 mg. of thallium sulfate was spread over the bait

used in the field control experiments. From estimates available on the dosage of thallium sulfate fatal to humans, the 30 mg. dosage appeared to be within a good margin of safety. Additional precautions were taken by coloring the poisoned bait green and placing it in a container which was clearly marked "Poison."

The bait container consisted of a one-quart oil can placed on its side with one lid bent outward horizontally to shield the open end from rain. The can was fastened to the ground with a 5-inch spike driven through the bottom. The poisoned bait was placed inside and against the back of the can. The use of the can minimized the risk of other animals reaching the poisoned bait and kept the bait dry in the field.

Three field control experiments were conducted, 2 in Puerto Rico and one on Pineros Island, near Puerto Rico. The experiments in Puerto Rico were conducted on a one-square-mile, heavily grassed area at Roosevelt Roads and on a 2-square-mile area at Sabana Llana. Bait stations were located in suitable mongoose habitats at the rate of one per acre, and were fewer than one per acre in the less suitable habitats. A 14-day poisoning period was used in both experiments. Before the poisoning operations began, 100 traps at Roosevelt Roads and 70 at Sabana Llana were operated for 3 successive days to obtain an index of the mongoose population. Thirty-five mongooses were trapped at Roosevelt Roads and 8 at Sabana Llana. The traps were closed and left in place during the poisoning period.

Following the prepoisoning trapping period, poisoned bait was placed in the bait stations. For the first three days an average of 57 and 18 per cent of the bait stations was visited at Roosevelt Roads and Sabana Llana, respectively. The visits to the bait stations decreased daily until at the 14th day only 2 per cent were visited at both Roosevelt Roads and Sabana Llana. Ants took a small percentage of the bait at Roosevelt Roads until the cans were treated with 10 per cent DDT. Eleven sick or dead mongooses were observed during the poisoning period at Roosevelt Roads and one dead mongoose was found at Sabana Llana. The odor of other dead animals was detected but they could not be located due to the dense tropical growth.

At the end of the 14-day poisoning period the traps were reset. During this second 3-day trapping period, 6 mongooses were trapped at Roosevelt Roads and one at Sabana Llana. Four of those trapped at Roosevelt Roads were healthy and were released again, while 2 appeared to be sick animals and were taken to the laboratory. One of the sick animals died within 24 hours, the other in 48 hours. Thus, counting only the healthy animals there was an indicated reduction of 89 per cent in the population of mongooses at Roosevelt Roads and 88 per cent at Sabana Llana. The control may have been better than the results indicated, because the healthy animals which were trapped at the end of the poisoning period may have migrated into the depleted area. The removal of mongooses from the test plots could have been an incentive for other mongooses from the surrounding areas to move in, since there were no natural barriers to prevent it. This increase very likely was more important than the possible decreased catch due to trap shyness.

The test area at Sabana Llana was typical of the general habitat in Puerto Rico. A campaign to advise residents in the area of the tests was staged before poison was placed in the field. The people were warned to keep their children away from the poison bait stations and to confine their dogs and cats for the 2-week poisoning period. Warning signs were located strategically in the area further to warn and to advise the residents. In spite of these precautions, 4 dogs, 3 cats, and 1 hen were found dead during the experiment. The cause of death of these animals was not determined, but it was assumed they died of thallium sulfate poisoning.

The Sabana Llana area was quite heavily infested with rats, which permitted an evaluation of the effects of the poisoning program on the rat population. Based on a prepoisoning trap catch of 18 rats and a postpoisoning catch of 4, there was an indicated reduction of 78 per cent in the rat population.

The experiment on Pineros Island was conducted to determine the possibility of exterminating the mongoose by the poison bait method. The Island lies 1½ miles from the northeast coast of Puerto Rico, and is approximately 1 mile wide by 1½ miles long. A fisherman was its sole inhabitant. The land has never been cultivated and is covered by dense tropical vegetation. Distances were misjudged because of the dense growth and bait stations were set at a rate of 1.5/acre instead of the desired one per acre. All other methods were similar to those used at Roosevelt Roads, except that the Island was divided into 3 areas for poisoning, each about ½ mile by 1 mile in size. The start of the poisoning was staggered over a 2-week period, beginning in the middle section a week after the bait had been set out in the western section and beginning in the eastern section 2 weeks after the start in the western section. Bait was renewed daily in the stations in the western section for 19 days and in the other two sections for 14 days. The stations were left in place and baited at the close of the poisoning period.

The poison bait consumption followed the same pattern as in the experiments at Roosevelt Roads and Sabana Llana, approaching zero at the end of the poisoning period. Thirty dead or sick mongooses were seen during the poisoning period. The odor of other decaying animals was detected, but these were not located for identification due to the dense jungle growth. Two dogs owned by the single Island resident were killed, apparently by the poison bait.

In a 2-day prepoisoning trapping period with 70 traps, 27 mongooses were trapped and released. In 7 days of postpoisoning trapping no mongooses were taken. Four months later, however, 6 adolescent mongooses were captured with 70 traps in 21 days of trapping. Failure to trap mongooses immediately following the poisoning period was probably due to the fact that only mongoose pups, too young to travel far from their burrows or too small to spring the traps, survived the poisoning operation. All adult mongooses on Pineros Island apparently were killed by the poisoning campaign.

The cost of controlling mongooses by the poison bait method was estimated at approximately 17 cents per acre for Puerto Rico and 76 cents per acre for Pineros Island. The higher cost for Pineros was due to the dense jungle growth, placement of 1.5 instead of one bait station per acre, and the added cost of transporting men and equipment to and from the Island. The cost of the poisoning for Puerto

Rico included labor and materials, as well as warning signs and the hire of a sound truck. This figure is far lower than the estimated cost of 57 cents per acre for trapping.

SUMMARY

Warfarin, red squill, barium carbonate, strychnine, ANTU, zinc phosphide, microfine arsenic trioxide, thallium sulfate, sodium fluoroacetate ("1080"), and ground glass were tested against a total of 208 mongooses. Only compound "1080" fed for one dose at 80 mg./kg., and thallium sulfate cumulatively fed at a dosage of 60 mg./kg. for three feedings were effective against the mongoose.

In two field control experiments in Puerto Rico, thallium sulfate on sun-dried fresh fish effected about a 90 per cent reduction of the mongoose population at an estimated cost of about 17 cents per acre. In another experiment on Pineros Island near Puerto Rico, all adult mongooses apparently were killed by the thallium sulfate poison bait method. Some very young animals survived, as indicated by trapping of several adolescent mongooses on the Island 4 months after the poisoning experiment.

ACKNOWLEDGMENTS

The author expresses his grateful appreciation to Dr. Juan A Pons, Commissioner, and Mr. Felix C. Garcia, Chief, Bureau of Vector Control, Commonwealth of Puerto Rico Department of Health, and to Captain W. C. Baty, MC, District Medical Officer, 10th Naval District, U. S. Navy, San Juan, Puerto Rico, for their interest and support.

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RESULTS OF LABORATORY SCREENING TESTS OF CHEMICAL COMPOUNDS FOR MOLLUSCICIDAL ACTIVITY

III. Derivatives of Abietic Acid

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In the course of the screening program being carried on in these Institutes for finding chemical agents suitable for use in the destruction of the snail vectors of the schistosomes parasitizing man, it was observed that Rosin Amine D acetate[1] was lethal to *Australorbis glabratus*. A number of compounds closely related to Rosin Amine D acetate accordingly were tested. Many of these were found to possess similar activity. This paper reports the results obtained with this group of compounds and also a number of the simpler amines.

After our manuscript had been completed, we came across an interesting article by Perlowagora-Szumlewicz and Almeida de Aguiar (1952) reporting "liquid rosin soap" to be a potential molluscicide. These authors found that under laboratory conditions "liquid rosin soap" at 10 ppm killed all snails within 48 hours of contact; at 15 ppm it was lethal within 30 hours and at 150 ppm within 10 hours. They indicated that the active principle in the rosin soap was abietic acid.

MATERIALS AND METHODS

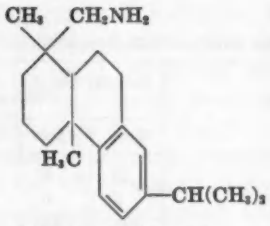
The details of the maintenance of the snail colonies and the experimental procedures employed have been described in Paper I of this series (Nolan, Bond and Mann, 1953). As before, initial tests of the chemicals were made at a concentration of 10 parts per million (ppm) for an exposure period of 24 hours, followed by a recovery period of 24 hours in fresh aerated water with food provided. Compounds which showed activity at 10 ppm were further tested at lower concentrations, usually 5, 3, and 1 ppm, to determine approximately the lower limit of effective concentrations. Control snails were maintained in untreated water for the same periods of time. There were no deaths among the controls.

The numbering of the compounds is a continuation of that in Papers I and II of the series. As in previous papers mortalities are expressed as the percentage of the snails which died during the exposure period and/or the recovery period.

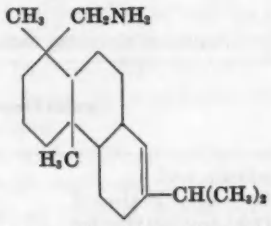
RESULTS AND DISCUSSION

Rosin Amine D is a mixture of primary amines, stated by the manufacturer to be composed approximately of 50 per cent dehydroabietylamine, 20 per cent dihydroabietylamine, 20 per cent tetrahydroabietylamine, and 10 per cent neutral oils. The raw material for the manufacture of Rosin Amine D is stated

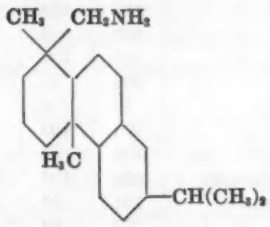
[1] A commercial product manufactured by the Hercules Powder Company, Wilmington, Delaware.



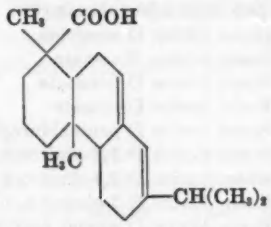
Dehydroabietylamine ($C_{20}H_{29}N$)



Dihydroabietylamine($C_{20}H_{31}N$)



Tetrahydroabietylamine ($C_{20}H_{37}N$)



Abietic acid ($C_{20}H_{30}O_2$)

to be a modified rosin; namely, a dehydrogenated-disproportionated rosin made from a refined wood rosin. Rosin is composed principally of abietic acid and/or its isomers. The preparation of Rosin Amine D involves a conversion of the carboxyl group of the resin acids to an aminomethyl group.

The presence of a primary amin group in Rosin Amine D makes possible the formation of salts, such as the acetate, benzoate, cumate, etc. Salts are also formed with those phenols that are sufficiently acidic due to the presence of substituents, *viz.*, 2,4-dinitrophenol; halogenated phenols; etc. Further, Rosin Amine D forms complexes with certain metallic salts, such as copper acetate. Examples of each type of compound were included in the tests.

The data obtained with the chemicals tested are recorded in Tables 1 and 2. From the data in Table 1, it can be seen that, whereas abietic acid and hydroabietyl alcohol were ineffective at 10 ppm, the various abietylamines were all very active, some at concentrations as low as 5 and 3 ppm. Activity in the abietylamines appears to increase with increased hydrogenation of the nucleus, being greatest with tetrahydroabietylamine. Rosin Amine D acetate at 5 ppm gave a higher kill of the snails than did Rosin Amine D emulsion at the same concentration. This result may well be due to the greater solubility of the acetate salt. The benzoate and the cumate salts contain less active principle than does the acetate, due to the higher molecular weight of the anion, hence, lower activities were obtained as was to be expected. Also, these salts are less soluble in water than the acetate. Acetic, benzoic, and cumic acids themselves were ineffective (Table 2).

Interpretation of the results obtained with the phenolic salts is complicated by the fact that the phenols themselves possess activity. No synergism is evident, however, since the results in every case are close to the values calculated for equimolar quantities of Rosin Amine D and the phenol. For purposes of com-

TABLE 1

Results of screening tests with abietic acid and its derivatives

Compound Number	Chemical Compound	Mortality (per cent) of *Australorbis glabratus* at:			
		10 ppm	5 ppm	3 ppm	1 ppm
729	Abietic acid	10	0	0	0
730	Hydroabietyl alcohol	0	0	0	0
731	Dehydroabietylamine	100	70	10	0
732	Dihydroabietylamine	100	100	70	0
733	Tetrahydroabietylamine	100	100	90	0
734	Rosin Amine D emulsion	100	0	10	0
735	Rosin Amine D acetate	100	100	0	0
736	Rosin Amine D benzoate	100	20	0	0
737	Rosin Amine D cumate	70	0	10	0
738	Rosin Amine D pentachlorophenate	100	100	50	0
739	Rosin Amine D 2,4-dinitrophenate	100	30	0	0
740	Rosin Amine D 2,4-dinitro-6-*sec*-butylphenate	100	100	60	0
741	Rosin Amine D 2-phenyl-4,6-dinitrophenate	100	90	40	10
743	Rosin Amine D copper complex	100	90	50	40
744	Rosin Amine D zinc dimethyldithiocarbamate	100	0	0	0

TABLE 2

Results of screening tests with related acids, phenols, and metal complexes

Compound Number	Chemical Compound	Mortality (per cent) of *Australorbis glabratus* at:			
		10 ppm	5 ppm	3 ppm	1 ppm
745	Acetic acid	0	0	0	0
746	Benzoic acid	0	—	—	—
747	Cumic acid	0	—	—	—
90	Pentachlorophenol	100	100	100	0
99	2,4-Dinitrophenol	0	—	—	—
238	2,4-Dinitro-6-*sec*-butylphenol	100	100	100	—
413	2-Phenyl-4,6-dinitrophenol	100	100	100	60
748	Copper acetate	100	90	40	10
749	Zinc dimethyldithiocarbamate*	100	100	100	70

* Zinc dimethyldithiocarbamate is a potential molluscicide. It and related compounds will be discussed more fully in a later paper.

parison, the results previously obtained with the corresponding phenols are included in Table 2.

To see if the abietyl nucleus is necessary for activity, a number of other amines were also tested. These were *n*-amylamine (No. 750); *n*-octylamine (751); cetylamine (752); ethanolamine (753); 1,10-decanediamine (754); *N*-(β-hydroxyethyl)ethylenediamine (755); tetraethylenepentamine (756); 2-aminoethyl sulfuric acid (757); α-aminocaprylic acid (758); cyclohexylamine (759); benzylamine (760); β-phenylethylamine (761); β-(3,4-dimethoxyphenyl)ethylamine (762); and 3,5-diiodotyrosine dihydrate (763). All of these compounds were in-

effective at 10 ppm. Unfortunately, neither cyclohexylmethylamine, 1-methylcyclohexylmethylamine, nor any closely related compounds were available for testing. These could conceivably possess high activity. Most certainly, the data obtained with them would be of considerable value in determining the seat of activity in Rosin Amine D and related compounds.

On the basis of the results obtained, Rosin Amine D and related compounds do not on the whole appear to possess as high molluscicidal activity as does pentachlorophenol, the molluscicide of choice at present. However, the ready commercial availability of the abietylamines at relatively low cost, plus the fact that compounds of this type are currently being used in large quantities as algicides in irrigation waters in the United States (Bowser, 1951), suggests that these compounds be given consideration as potential molluscicides.

SUMMARY

Abietic acid and 14 related compounds were screened for activity against *Australorbis glabratus*. High activity at 10 ppm was found among abietylamines and among Rosin Amine D and its salts. Fourteen simpler amines were found to be devoid of activity at 10 ppm.

The seat of activity in the abietic nucleus remains uncertain.

ACKNOWLEDGMENTS

The writers are indebted to Elizabeth M. Landry and Lawrence C. Pulley for technical assistance. Especial thanks for supplying chemical compounds are extended to Chemical-Biological Coordination Center (Nos. 754, 758, and 763); Hercules Powder Company (Nos. 730–741, 743, 744, and 747); Monsanto Chemical Company (Nos. 749, 761, and 762); and Parke, Davis and Company (Nos. 755 and 756).

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Hugh Roy Cullen, A Story of American Opportunity. by Ed Kilman and Theon Wright. 375 pp., illustrated. New York: Prentice-Hall, Inc. 1954.

BOOK REVIEWS

Protozoölogy, by RICHARD R. KUDO, D.SC., Professor of Zoology, University of Illinois, Urbana, Illinois. Fourth edition. 966 pp. with 376 illustrations, including 4 in color. Springfield, Illinois: Charles C. Thomas, Publisher, 1954. Price $10.75.

The publication of the considerably enlarged and revised fourth edition of this standard text on Protozoölogy is an indication of general endorsement of its usefulness. While it continues to be the only modern, single volume presenting basic information on protozoa for teaching the subject to advanced undergraduate and graduate students, it now becomes, in its expanded form, a valuable reference manual for investigators in this field of zoölogy.

As in previous editions, the present one is divided into two main parts, *viz.*, *General Biology* (of the phylum Protozoa), and *Taxonomic and Special Biology*. Part I includes separate chapters on orientation, ecology, morphology, physiology, reproduction, and variation and heredity. The author is particularly skilled in his presentation of the morphologic characteristics and reproductive capacities of representative protozoa. In contrast, the subject of physiology is not as fully considered; this is a serious defect in the balance of material, in view of the modern trends towards exploration of the metabolic patterns of living organisms.

The subject of taxonomy is adequately and satisfactorily considered, employing the recognized conservative classification of the phylum, and including descriptions of most species of each genus, all relatively brief but adequate for species determination. In this section the reader is able to appreciate the relative number of recognized species of free-living and parasitic forms and to weigh the importance of each in the economy of the natural world.

Of special interest to medical parasitologists is the inclusion of *Toxoplasma* in the family Babesiidae of the Haemosporidia (page 625). Most students of *Toxosplama* have been unable to decide the exact relationship of this medically important protozoön.

The Volume is provided with well documented author sources in the text and representative references at the end of each chapter. Most of these antedate 1951 and the reviewer has discovered none later than 1952. There is an abundance of accurate, artistically executed illustrations throughout the text; these constitute valuable supplements to the textual descriptions of the numerous species.

The last chapter provides brief information on "Collection, Cultivation and Observation of Protozoa." There are comprehensive Author and Subject Indexes. Typographic errors are very few.

The volume is printed on white Lexicon English Finish paper, admirably adapted to the clear reproduction of both illustrations and text. It is attractively bound.

Both author and publisher are to be congratulated on the new edition, which is certain to find a place in the classroom and library of all persons who are interested in the subject of protozoölogy.

ERNEST CARROLL FAUST

Handbook of Tropical Dermatology and Medical Mycology, Volume II, including separately bound Appendix on Fundamental Dermatological Terminology, by R. D. G. PH. SIMONS, (ed.), Senior Lecturer at the Dermatological Clinic of the University of Leyden, and 58 Collaborators, 1705 pp. Amsterdam, Houston, New York, London: Elsevier Press, 1953. Price $15.00.

Volume II of this Handbook is an excellent comprehensive collaboration of 58 expert investigators who are authorities in their respective fields. It includes sections dealing with diseases due to animal parasites, allergy, malnutrition, metabolic disturbances and tumors of the skin. There are additional chapters on a small group of miscellaneous dermatoses such as ainhum, and a chapter which briefly reviews the tropical diseases of the skin likely to be encountered by a ship's surgeon. An interesting, clearly written and fairly comprehensive review of the present knowledge of porphyria is included in the book.

Of special interest is the chapter on Clinical and Biochemical Features of the Skin in Malnutrition. The author summarizes in outline form the known facts concerning the biochemistry of the skin and then correlates in so far as it is possible to do so, the changes in the skin which take place in dietary deficiency states. About half of this volume is concerned with a comprehensive survey of diseases due to fungi. This section is illustrated with a large number of photographs, both clinical and laboratory, which do much to increase the value of the text. Those who are called upon to be occasional mycologists will find the chapters of Mycological Technique and on the Cultural Characteristics of saprophytic fungi to be especially helpful.

Simons concludes the volume with an "Epilogue in the Jungle of Dermatology" in which he appeals for an experimental crusade to clear medicine of its confusing array of "hearsay" science and medical superstition.

As with the first volume, commendation is due editor Simons for the excellence of the translation. I have no hesitancy in recommending this "Handbook" most highly as a reference for dermatologists, general practitioners and medical students interested in tropical medicine and medical mycology.

CHARLES R. REIN

Antibiotics Annual 1953–54. (Proceedings of the Symposium on Antibiotics), 632 pages, with index. HENRY WELCH, Ph.D., Chairman. Medical Encyclopedia, Inc., New York, 1953.

This book is a record of a symposium held under the auspices of the U. S. Department of Health, Education and Welfare, Food and Drug Administration, Division of Antibiotics during October 28, 29 and 30, 1953, in Washington, D. C. Dr. Welch is Director of the Federal Division of Antibiotics, Food and Drug Administration. It is a current record of the "antibiotic era" and accomplishments of the past year. In the introduction, Dr. Welch notes the important economic contribution made through the use of antibiotics, not only to man but to domestic animals and livestock as well. Newer antibiotics also have created problems of human sensitivity and the development of strains of micro-organisms which are drug-resistant. Continued research is directed toward their solution as well as developments in newer uses of recently introduced antibiotics.

Dr. Felix Marti-Ibanez, International Editor of *Antibiotics and Chemotherapy*, in a Section on an "Historical Perspective of Antibiotics—Past and Present" states his belief that we are only beginning to experience the influence of these drugs in medicine. Nothing has revolutionized therapeutics so profoundly, since these agents represent a new concept in the art of healing.

Because of the multi-dimensional character of the reports included in this symposium, only by consulting this record can the interested reader gain an appreciation of the complexity of the subjects included in the Annual.

HAMILTON H. ANDERSON

"A Manual of Tropical Medicine" by THOMAS T. MACKIE, M.D., Col., M.C., U.S.A. (Retired); GEORGE W. HUNTER, III, Ph.D., Col., M.S.C., U.S.A.; C. BROOKE WORTH, M.D., 2nd edition: 907 pp., 304 illustrations with 7 in color. Philadelphia & Lond., W. B. Saunders, 1954. Price $12.00.

The first edition of the Manual of Tropical Medicine was published during World War II under the auspices of the National Research Council to meet the needs of the armed forces in the tropics. The primary objective of the Manual was a concise presentation of the important aspects of the most significant tropical diseases. In the preparation of the second edition the authors have adhered to the fundamental objectives of the original volume. They have capitalized on the vast amount of data accumulated during the past ten years as a result of the stimulation to clinical and laboratory research imparted by the necessities and experiences of World War II. The second edition exceeds by more than 200 pages the length of the original Manual and in many fields there have been deletions and substitutions in the original text and many sections have been largely or entirely rewritten. The collaborators are distinguished in their respective fields and the presentations represent the view of recognized authorities. The text is extremely suitable to the needs of medical students, clinicians and medical officers in the tropics and this second edition therefore can be considered an excellent text rather than a Manual which ordinarily conveys limited implications. The illustrations, figures and tables are excellent. The treatment in general is up-to-date and quite definitive. There are few errors (the concentration of tartar emetic used in the treatment of schistosomiasis is given as 5% instead of 0.5%). The section on methods and procedures at the end of the text and the special section on the drug therapy of helminth infections should be very useful to clinicians and laboratory workers. The text dealing with the treatment of amebiasis and malaria is excellent and the tabular presentation of the drugs used in the treatment of amebiasis should be very useful. Since the usefulness of the text for medical officers is emphasized it is unfortunate that much of the clinical experience gained during World War II in such fields as schistosomiasis and leishmaniasis is not presented. Apart from this limited consideration the second edition of "A Manual of Tropical Medicine" should prove to be a convenient and useful reference book for students and clinicians.

H. MOST

The Laboratory Diagnosis of Leptospirosis, by J. W. WOLFF, M.D., Institute of Tropical Hygiene, Royal Tropical Institute, Amsterdam, The Netherlands. 99 pp., illustrated. Springfield, Illinois: Charles C. Thomas, 1954. Price $3.75.

This is a much needed text by a world-recognized authority on the leptospiroses. It is a monograph in one of the series of "American lectures in tests and techniques." It is printed in easy-to-read type on good paper and is attractively bound in a size that can readily be kept in a laboratory, where it will find its greatest usage.

The table of contents reveals sections on morphology and staining methods, diagnostic procedures including dark field examination, cultural procedures and media, animal inoculation and serologic procedures. Two sections are devoted to antigenic structures on leptospires and characteristics of type strains. These two chapters are especially important to all interested in leptospirosis.

The text is well organized and carefully written. The technics described are those that were developed by Dutch workers in the Netherlands and Indonesia over a period of twenty years. In general, the technics parallel those used by the American laboratories actively engaged in leptospiral research. There are, however, a few minor differences. Standard pipettes and an arithmetic progressive dilution scheme are generally used here, in place of the Pasteur pipettes and porcelain plates that Wolff describes. This is, however, only a difference in methodology. A difference in interpretation of agglutination-lysis results on serums does exist. As Borg-Petersen points out, absorption tests on patient's serum may not identify the infecting serotypes.

The author notes that leptospires do not survive in acid urine. There is some indication

that leptospires remain viable at pH's 5–5.5 for considerably longer periods of time than formerly believed. Perhaps the author should have given more consideration to other serologic procedures, such as the complement fixation technics. Illustrations of agglutination and lysis reactions would enhance the value of the description of the agglutination-lysis test. This volume will fill a very marked need in the diagnostic laboratory and in leptospiral research in general.

CHESTER A. GLEISER

The Dynamics of Virus and Rickettsial Infections. International Symposium. (Sponsored by Henry Ford Hospital, Detroit, Oct. 21–23, 1953.) Editors: FRANK W. HARTMAN, FRANK L. HORSFALL, JR., JOHN G. KIDD. 461 pp. illustrated. New York, The Blakiston Co., 1954. Price $7.50.

To those engaged in the field of virology, this book will serve to corroborate the realization that virology has emerged at last from the growing pains of adolescence and has come of age, and to those working in allied sciences, even a general perusal of this volume will provide convincing evidence that the virologist has not only come into his own but he is making revealing contributions and propounding fundamental concepts which now touch many disciplines.

This book constitutes the proceedings of an International Symposium on the Dynamics of Virus and Rickettsial Infections held in Detroit, Michigan, October 21–23, 1953, at the Henry Ford Hospital. Thirty-three investigators, six of whom came from abroad, were invited to present papers on the results of their recent studies in the three major divisions of the virus field: animal, bacterial, and plant.

The material presented is largely a summary and appraisal of current knowledge on virus and rickettsial infections and an evaluation of the newly emerging concepts resulting from recent studies of the authors and their colleagues. This should have theoretical interest for the scientist but there is much of practical value for the physician and public health worker as well.

Among the problems presented and discussed in a most informative and stimulating manner are the mechanisms of virus reproduction and the interplay of surface interactions, enzymes, nucleic acids, proteins, and cellular metabolism in the adsorption, penetration and replication of virus particles within the host cells. Studies on double infections, genetic recombination and mutation of viruses are also described and critically appraised.

Current thinking on ecology and pathogenesis emphasizes that although in virus diseases the importance of reservoir hosts and efficient transmitters is well established, knowledge concerning the entire ecologic cycle is only incomplete in most instances. Population fluctuations of the vectors, hosts, and pathogenic agents are affected by environmental factors which change with deforestation and human settlement, and the role of the immune state and the healthy carrier in the transmission chain may explain the different epidemiologic pictures observed.

The mechanisms of convalescent immunity and how it may be artificially simulated and the induction of immunity by virulent inactivated and avirulent live virus vaccines, particularly with regard to their application to poliomyelitis, receive timely consideration, although conclusive answers to many controversial questions cannot be given.

The discussions on the laboratory diagnosis of virus diseases of man have special interest. The recovery and identification of virus and demonstration of specific antibody response are still prerequisite for diagnosis. The disadvantages of the expensive and time-consuming methods involved, the obtaining of results frequently in retrospect, and other limitations have made the practical application of these procedures questionable in the opinion of some of the participants. With the exception of smallpox, technics for the rapid diagnosis of virus diseases have been devised in relatively few instances. However, tissue cultures are currently replacing animals as the medium for isolation and identification of viruses and the detection of specific antibodies.

With the exception of the rickettsiae and viruses of the psittacosis-lymphogranuloma

venereum group, chemotherapeutic agents effective in combating virus infections are still lacking. There are, however, a number of substances which inhibit the multiplication of certain animal, plant, and bacterial viruses under experimental conditions. Among these substances are proflavine, various antimetabolites, derivatives of benzimidazole and naturally occurring substances such as polysaccharide derived from *K. pneumoniae*, and apple pectin. The mechanic and kinetic aspects of these inhibitory reactions have not been widely studied and are as yet poorly understood but it is evident from the material presented that sufficient delving into these areas of investigation will uncover substances of academic and practical use.

As with all good works, this book is not without its imperfections of omission and commission, but this is reading matter that should provide satisfaction and profit to all and, in addition, serve as a provocative guide into areas of research that deserve further exploration.

MORRIS SCHAEFFER

The Meaning of Social Medicine, by IAGO GALDSTON, Secretary, Medical Information Bureau, The New York Academy of Medicine. 137 pp. Cambridge, Mass.: Published for the Commonwealth Fund by the Harvard University Press, 1954. Price $2.75. (Distributed in Great Britain by Oxford University Press.)

This is the most comprehensive exposition of an as yet undefined medical discipline that has yet appeared and consequently is a must for all interested in the field. The purpose of the book is to advance and support the conclusions in the final chapter, "Social Medicine and Medical Education." The author leads up to this by a number of expository chapters, beginning with a review, and comments on some of the varying definitions of social medicine. Unfortunately, he does not, himself, advance a definition except by inference through stating that "the foundation of social medicine must be a broad and inclusive dynamic and developmental biology." Social pathology and biostatistics would serve to indicate where and how biological requirements have not been met. The present "etiological medicine" is criticized because while it has achieved much in the treatment and prevention of disease it has failed in the elimination of disease and the promotion of health. Conventional medical education is thus criticized as too slavishly recapitulating therapeutic medicine and thereby committing the practitioner to a negative outlook because it has not kept pace with the "ideological growth of the biological sciences." Consequently "the physician is becoming increasingly a middleman between the patient and the diagnostic laboratory on the one hand and the pharmaceutical house on the other." The laboratory makes the diagnosis; the pharmaceutical house provides the treatment. Galdston maintains that social medicine cannot be fitted into medical education as now constituted. While social medicine is not fusible with curative medicine, the author affirms that social medicine can absorb curative medicine. Unfortunately at this point the reader is left speculating as to how the new medical curriculum would specifically be designed, as it is outlined only in the broadest terms of revolving around "growth, development, change, decline, and all the vast numbers of experiential instances that are summated under these larger headings."

However, as indicated, the preliminary chapters are thought provoking. Social medicine, as distinct from socialized medicine, was initiated in England and there is an interesting addendum, "Social Medicine in England," reviewing its development and present status. The latter confirms the emergent position of social medicine itself. The comprehensive bibliography adds much to the value of the book.

JOHN B. GRANT

The Science Book of Wonder Drugs, by DONALD G. COOLEY. 247 pp., illustrated. New York, Franklin Watts, Inc. 1954. Price $2.95.

Mr. Donald G. Cooley is among the ablest of those middlemen who bring complex sciences within reach of ordinary people. He discusses, in an entertaining way, one of the most exciting frontiers that science has ever explored. The site of this exploration is the bio-

chemical world within the human organism—a place of overpowering minuteness, intricacy and busyness. Man's body, Mr. Cooley reports, is composed of 26 trillion 500 billion cells, each with its own universe of activity. Not one cell is ever at rest. Day and night they are moving ceaselessly in chains of chemical inter-relationships. These myriads of agitated flecks manage, in some inscrutable way, to unite into one community called man's body. But they are not allowed to live in peace. Even while man preaches peace and brotherhood, billions of bacteria, fungi, molds, viruses and protozoa within him are at the throats of billions of fellow organisms in a fight to the finish for *lebensraum*.

Into this maelstrom the wonder drugs are sent with orders to help friend and injure foe. They carry out this incredible mission with a biochemical cunning far superior to the military cunning of man. They strengthen ally, starve enemy, deceive, disrupt and make the sudden ruthless attack.

The success of the new chemical magic marks the most dramatic era in medical history. The author tells us that during the brief period it has been operating (1935–1954) life expectancy in the U. S. increased ten years; school absences decreased by half; 40 per cent of infectious diseases under medical care were controlled—some almost eliminated—with the promise that others may soon follow. The list is impressive; the amount of drugs involved tremendous. We use 3,200 tons of sulfa and 320 tons of penicillin annually.

Encouraging discoveries continue to pour from biochemical laboratories. From the many findings which the author mentions, a few will indicate the varied directions of research. Rats can be changed from drunkards to teetotalers by altering the vitamin content of their diets; a hog, fed minute amounts of antibiotics, can be fattened on 40 lbs. less feed, thus saving 2,000,000 tons of produce each year; eunuchs do not get cancer; a virus may have three or more parents instead of the humdrum two.

There are dangers, too, in the use of wonder drugs; of 300 known antibiotics, not more than 12 work safely. But with thousands yet untried, Mr. Cooley predicts a future for them even more spectacular than their past. Whether a perfected chemotherapy will eliminate in time all human ills—even cancer, insanity and the debilities of old age—is none too certain, but at any rate this is a book which physicians can recommend to lay friends who would like to experience the thrills of scientific adventure and prediction.

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"At the present time chloramphenicol [CHLOROMYCETIN] is recognized as a potent antibiotic whose ease of administration and prompt diffusion into serum and spinal fluid makes it a particularly useful agent in the treatment of many forms of purulent meningitis."[3]

in bacterial endocarditis

"Within ten days [after therapy with CHLOROMYCETIN was begun] there was a dramatic improvement in the patient's clinical appearance and the sedimentation rate and temperature became normal."[4]

in rickettsial diseases

"Chloramphenicol [CHLOROMYCETIN] has been used with striking success in patients with scrub typhus, murine typhus, Rocky Mountain spotted fever, and epidemic typhus."[5]

Chloromycetin®

(Chloramphenicol, Parke-Davis)

CHLOROMYCETIN is a potent therapeutic agent and, because certain blood dyscrasias have been associated with its administration, it should not be used indiscriminately or for minor infections. Furthermore, as with certain other drugs, adequate blood studies should be made when the patient requires prolonged or intermittent therapy.

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3. Hanbery, J. W.: *Neurology* 4:301, 1954.
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PARKE, DAVIS & COMPANY DETROIT 32, MICHIGAN

DIFCO

Diagnostic Reagents
for the Determination of

ANTISTREPTOLYSIN TITERS

Bacto-Streptolysin O Reagent is a standardized reagent recommended as an aid in the diagnosis of Rheumatic Fever, Glomerulonephritis and other diseases associated with Group A streptococcal infections.

SENSITIVITY TESTS

The significance of determining bacterial sensitivity is accentuated by the discovery of increasing numbers of antibiotics, sulfonamides, and other chemotherapeutic agents and the development of resistant organisms to one or more of these compounds. Sensitivity or resistance is easily determined with Bacto-Sensitivity Disks.

HETEROPHILE ANTIBODIES

The differential diagnosis of Infectious Mononucleosis requires the use of standardized heterophile reagents:

Bacto-Guinea Pig Kidney Antigen, Desiccated and Suspension
Bacto-Beef Cell Antigen, Desiccated and Suspension

BLOOD COAGULATION

Prothrombin determinations require accurately prepared interstandardized reagents:

Bacto-Thromboplastin
Bacto-Prothrombin 2 Stage Reagent
Bacto-Prothrombin Free Beef Plasma
Bacto-Prothrombin Free Rabbit Plasma
Bacto-AC Globulin
Bacto-Sodium Chloride 0.85 Per Cent
Bacto-Sodium Oxalate Solution 0.1 Molar
Bacto-Calcium Chloride Solution 0.02 Molar

LIVER PATHOLOGY

Differential diagnosis of hepatic disturbances is aided by standardized reagents:

Bacto-Cephalin Cholesterol Antigen
Bacto-Thymol Turbidity Reagent
Bacto-Kingsbury Turbidity Standards
Bacto-Thromboplastin and other Prothrombin Reagents

RENAL FUNCTION

Phenolsulfonphthalein Ampules, Difco is a carefully prepared injectable recommended for use in determining renal excretion rate.

HEMAGGLUTINATION OF RED BLOOD CELLS

Accurately determined by the use of:

Bacto-Trypsin 1% for Hemagglutination
Bacto-Hemagglutination Buffer
Trypsin, Difco, 1:250
Bacto-Bovine Albumin 30%
Bacto-Phytohemagglutinin

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